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SOROSPORELLA UVELLA AND ITS OCCURRENCE IN CUTWORMS IN AMERICA

[PRELIMINARY PAPER]

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In 1888 Sorokin (5)[1] described and illustrated as a parasite of the cutworm *Agrotis segetum* Schiff., in Russia, a fungus which he called *Sorosporella agrotidis*, employing thereby a new generic as well as a new specific

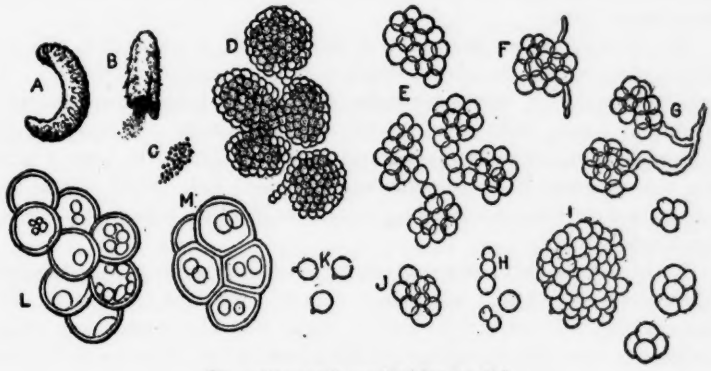

FIG. 1.—*Sorosporella agrotidis* (after Sorokin).

name. The descriptive generic name was chosen because of the presence in infected insects of grapelike masses of spores, masses which reminded Sorokin of similar formations in the genus Sorosporium of the Ustilaginales, although he suggested no relationship between the two forms. For purposes of comparison, a copy of Sorokin's illustration is presented (fig. 1) which, together with his diagnosis, is sufficiently complete to render an identification of the organism possible.

Sorokin had but few diseased larvæ, and did not, therefore, attempt inoculation experiments; nor did he study at any length the life history

[1] Reference is made by number to "Literature cited," p. 194.

of the organism. As a consequence, subsequent writers have been at a loss as to the systematic position of the genus Sorosporella. Several have gone so far as to suggest its affinity to the Entomophthorales, basing their claims on its apparent resemblance to the genus Massospora Peck, which Forbes and Thaxter have shown to be entomophthoraceous.

In 1889 Giard (2) translated Sorokin's article into French, and in the same issue of the publication followed it by a note in which he pointed out that *Sorosporella agrotidis* Sorokin was undoubtedly identical with *Tarichium uvella* Krassilstschik. Giard reached this conclusion because of the many points of similarity between the two forms, such, for example, as the reddish color of the fungus mass when observed with the naked eye, the peculiar internal development of both forms, the comparative uniformity of the spores in form and size (Sorokin, 4 to 7μ; Krassilstschik, 8 to 10μ), and the existence of papillæ on the spores. He furthermore called attention to the fact that the specific name "*uvella*" of Krassilstschik served also to indicate this peculiar massing of spores, a condition entirely dissimilar to that found in other entomogenous forms.

The generic name Tarichium, if valid at all, is applicable to those forms of Entomophthorales in which resting spores only are known. As will be shown later, the fungus under consideration is in no way connected with this group, and, therefore, the use of the name Tarichium as employed by Krassilstschik (4) becomes invalid. Hence, the correct name for the organism is *Sorosporella uvella* (Krass.) Gd., Giard having first recognized the form described by Sorokin as identical with that described by Krassilstschik.

Early in June two larvæ and one pupa of a cutworm (*Euxoa tessellata* Harris) which had died in breeding jars were given to the writer. The insects originally came from College Park, Md. No fungus was visible externally, but upon breaking open the larvæ (Pl. 66, *A*) a reddish brown powdery spore mass was seen which completely filled the interior of the insect's body. Microscopic examination demonstrated that the fungus was none other than *Sorosporella uvella* (Krass.) Gd.

So far as the writer is aware, there are no records of the appearance of this fungus in Europe since 1888; and, furthermore, this seems to be the first published account of its occurrence in America, except for a brief note of its presence in Ottawa, Canada, by Gibson (1).

The object of the present preliminary note is merely to record the presence of this entomogenous form in the United States and to present some evidence to show that it is not entomophthoraceous, deferring for a time a more complete account of its life history as well as a consideration of its efficacy as a fungus parasite of insects.

In the discussion which follows, the large, spherical, thick-walled cells will be spoken of as spores and the aggregations of these cells as spore masses. These terms have been used advisedly. It is realized that so

long as the method of formation of these bodies is unknown such usage is not entirely justified. At the present time, however, these names are perhaps as convenient and acceptable as any.

As noted above, the vegetative development of the organism is apparently completed within the body of the insect, the growth almost wholly replacing the body contents. Under normal conditions, the chitinous body wall of the insect remains intact, becoming dry and brittle, translucent, and very much wrinkled. The body does not, however, become mummified as do the bodies of larvæ infected with species of Cordyceps. On the contrary it is loosely filled with quantities of spores and resembles a minute sac filled with dust.

The fungus consists of large irregular masses (Pl. 66, *B*, *C*), which in turn are composed of numerous spherical sporelike elements that are firmly attached to one another. These spore aggregations vary greatly in size and form, but the elements of which they are composed are quite uniform in size and structure. Upon examining a large number of crushed spore masses, however, it would appear at times that the peripheral cells were, on the whole, somewhat larger than the inner ones, but at the present time it is not possible to say that this difference invariably exists. A further difference exists in the germinative power of the outer and inner cells. This condition may be readily seen if a crushed spore mass is mounted in a hanging drop of water. Those cells or pseudospores which were originally internal remain inactive, while those spores which were external produce germ tubes in due time. In other respects the individual elements are similar. They are nearly spherical in form, have rather thick walls, are colorless when viewed individually, and measure from 7 to 10μ in diameter.

As already stated, the spores can be separated from one another only with great difficulty, and on crushing a spore mass ruptured spore walls are often seen attached to uninjured spores (Pl. 66, *E*, *a*).

Sorokin and Krassilstschik both speak of the presence of papillæ on the spores, and Sorokin also observed a mycelium in connection with the spore masses (fig. 1). The same author suggested that the papillæ were peduncles to which the mycelial filaments had been attached, yet thus far no fungus mycelium has been seen within the insect's body, although papillæ have occasionally been observed (Pl. 66, *E*, *b*). At the present time, therefore, the manner of formation of the spore masses is unknown, but as no mycelial filaments have been observed, and as the above-mentioned papillæ have been seen, though rarely, it seems probable that the spore masses may arise through the successive budding of the original elements. It will be necessary, however, to examine cutworm larvæ in early stages of the disease or to obtain some evidence by artificial culture before this point can be proved.

Up to this point, the writer's observations confirm those of Sorokin, who was, however, unable to pursue the study further, owing to lack of

material. Krassilstschik, according to Thaxter (7, p. 190) in his description of *Tarichium uvella*, states that in nutrient fluid the large spherical spores germinate and give rise to septate hyphæ that ultimately produce single, terminal, cylindrical colorless spores, measuring 9 by 3μ. Although Krassilstschik's description is meager and unaccompanied by figures, it would seem that this process agrees essentially with that described below.

Several more infected insects were received in July, so that enough material was available to conduct germination and cultural tests. At the end of 48 hours in hanging-drop water cultures each of the peripheral cells of the spore masses gives rise to one germ tube. These are at first simple, sometimes septate, and on the third or fourth day each produces a single cylindrical spore at its tip (Pl. 66, *G*). These spores, which may be called secondary spores, are entirely unlike those from which they arose, being colorless, thin walled, and cylindrical. Their measurements vary from 4 to 6μ in width and 9 to 11μ in length. Two large vacuoles are invariably present, one at each end of the spore. The germ tubes gradually lengthen somewhat and branch sparingly. The ultimate branches are often terminated by bottle-shaped portions that show, or at least have a tendency toward, verticillate grouping. At the end of 10 days growth ceases, owing apparently to the exhaustion of the nourishment supplied by the large spherical spores. During the latter part of this period the secondary spores are cut off successively from the tips of the sporophores, and in hanging-drop water cultures adhere to one another after abjunction (Pl. 66, *H*).

The promycelium-like sporiferous filaments arising from the large spherical cells are irregularly septate, limited in growth, and measure from 3 to 5μ in diameter.

From the above description it will be seen that the fungus under consideration has no characters in common with the Entomophthorales. The cœnocytic elements of growth of the latter, together with the method of spore formation and discharge, are in no way comparable to analogous processes in Sorosporella.

It is not at all certain whether the inner cells of the spore masses function as reservoirs of food for the outer germinating spores, as is supposed to be the case in some bulbils, because, judging from their staining properties after several days in hanging drops of water, there is apparently no reduction in the amount of their cell contents. Furthermore, while the individual elements are firmly fixed to one another, a connecting passage between them, although perhaps not necessary for the transfer of protoplasmic material, has yet to be demonstrated.

Attempts to cultivate the organism have not thus far yielded entirely satisfactory results, probably owing to an improper choice of nutrients. In the present preliminary trials only potato agar and oat agar were

employed, in both of which growth is exasperatingly slow. The outer cells of the spore masses, however, when plated in such media, give rise to a mycelium a portion of which is represented on Plate 66, *N*. No spores have as yet developed in such cultures, although they are now several weeks old.

No attempts have been made to study the development of the secondary spores or conidia in culture media, but their germination in water has been observed (Pl. 66, *M*, *O*).

From the studies thus far conducted it seems probable that the large, spherical, thick-walled cells function as resting spores, which in this fungus are closely attached to one another in masses. Such a condition is, as noted by Sorokin, very similar to that found in certain smuts, such as Urocystis and Tubercinia.

The early stages of germination of the spore balls of such smuts and of the spore masses of Sorosporella are also similar. The later development of the germinating spore masses of Sorosporella, however, more closely resembles that of the verticillate Hyphomycetes (Pl. 66, *H*, *K*, *L*). The branched sporophores ending in bottle-shaped tips and the abjunction of the typical conidia which adhere to one another in groups as they are cut off suggest this relationship.

While the spore masses, as mentioned above, bear a certain superficial resemblance to the spore balls of Ustilaginales, they nevertheless more closely resemble that class of propagative bodies known as bulbils, which are known to occur in the life histories of certain Ascomycetes and Basidiomycetes.

Hotson (3) and others have shown that bulbils are often directly associated with hyphomycetous growths. In some cases, as in *Papulospora aspergilliformis* as figured by Hotson, the bulbils upon germination give rise to typical hyphomycetous forms. In other cases the same mycelium produces bulbils and conidia at different places.

What the early stages in the development of Sorosporella are in the insect is not known. The presence of papillæ on the spores suggests that vegetative development is carried on by a budding process, but, as mentioned above, this point has yet to be proved. It is known, however, that finally the entire larval body is filled with the above-described large spore aggregations, which will undoubtedly be incorporated in the soil when the larvæ disintegrate. In a moist chamber such spore aggregations give rise to the secondary spores mentioned above, and it may be assumed that the same process takes place in the soil. In such a position the secondary spores will readily be able to infect fresh cutworms, which habitually burrow in the earth.

Thus far no experiments have been conducted to test the parasitism of *Sorosporella uvella*, but it is hoped that these experiments, as well as a more complete study of the life history of the organism, will be accomplished soon.

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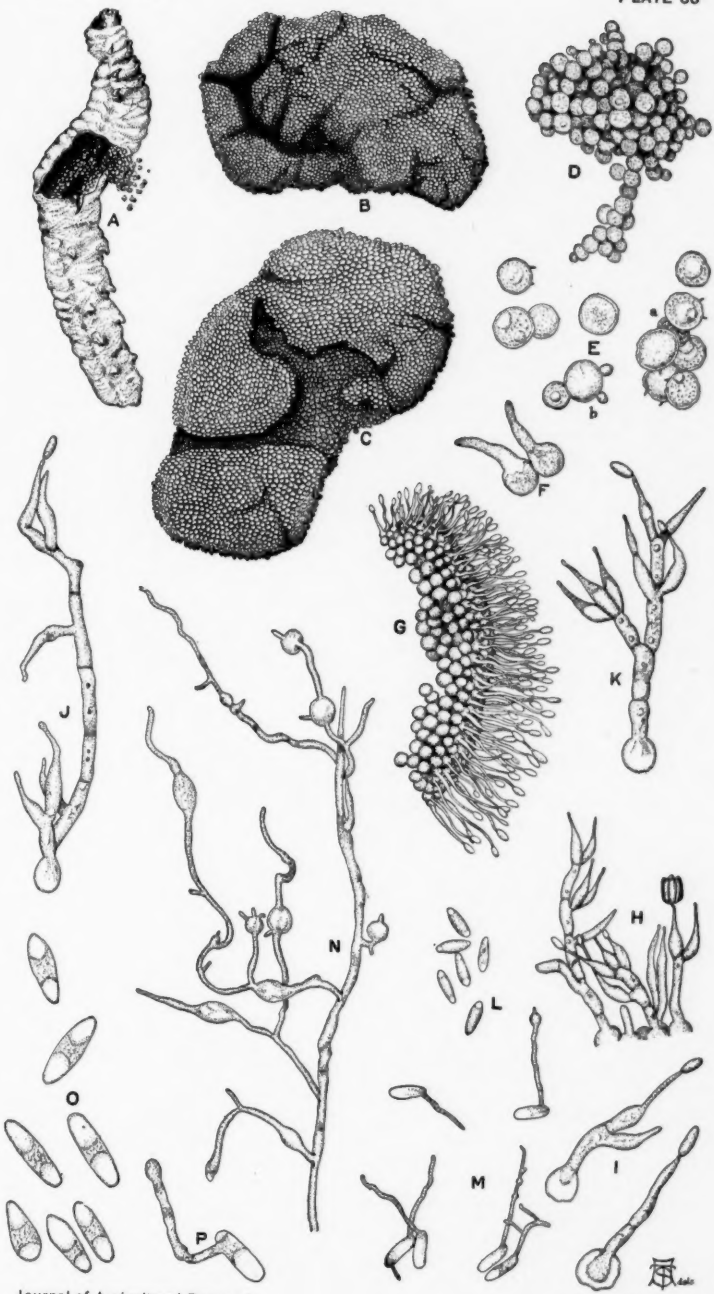
PLATE 66

Sorosporella uvella.

A.—Larva of *Euxoa tessellata* infected with *Sorosporella uvella*. ×1.2.
B, C.—Spore masses. ×120.
D.—A portion of a spore mass. ×252.8.
E.—*a*, Spores with ruptured walls of other spores attached. *b*, Spores showing papillæ. ×580.
F.—Germinating spores. ×580.
G.—A portion of a spore mass showing peripheral germination. ×252.8.
H, K.—Spores germinating, in part showing verticillate branching. ×580.
L.—Secondary spores. ×580.
M.—Secondary spores germinating. ×580.
N.—A portion of vegetative mycelium from a culture. ×580.
O.—Secondary spores. ×1080.
P.—Secondary spores germinating. ×1080.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MEASUREMENT OF THE INACTIVE, OR UNFREE, MOISTURE IN THE SOIL BY MEANS OF THE DILATOMETER METHOD

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INTRODUCTION

One of the problems in the domain of soil physics which still awaits a satisfactory and comprehensive solution is the relation that exists between the soil and the soil water. In this relation there are included two important and fundamental questions: (1) The effect of the soil upon the soil moisture and (2) the condition in which the moisture exists in the soil.

In the present paper there are presented two methods—the freezing-point and the dilatometer methods—which appear to give very valuable information concerning these two important and fundamental questions. The freezing-point method yields only qualitative results, while the dilatometer method gives quantitative data.

HISTORICAL REVIEW

In determining the lowering of the freezing point of soils (2) [1] by the freezing-point method at very low and very high moisture content it was found that in all soils, with the exception of quartz sand and some extreme types of sand, the lowering of the freezing point increased at a far greater rate than the percentage of water decreased. In other words, the ratio of the freeezing-point depression and the percentage of water content were not inversely proportional (approximately) as might be expected, save only in the quartz sand and some of the extreme types of sand. Thus, a clay at 92.76 per cent of moisture gave a depression of the freezing point of 0.039° C. and at 39.28 per cent 1.075° C. The ratio of the percentage of water at the two moisture contents is only 2.37, while that of the depression is 27.56.

This phenomenon was explained on the supposition that some of the water contained by the soils was either physically adsorbed or loosely chemically combined or both, in which event this portion of the water was not free or active to act as a solvent but was removed from the liquid phase and thus also from the field of action as far as the freezing-point lowering is concerned. Under this assumption the unusual results obtained could be easily explained. Thus, if a clay, for example, causes

[1] Reference is made by number to "Literature cited," p. 217.

Journal of Agricultural Research,
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Key No. Mich.—5

15 per cent of the water to become inactive or unfree either physically or chemically, or both, and at 39 per cent of moisture this clay gives a depression of 0.075° and at 22 per cent, 0.987° C., then in the first case there is 24 per cent of moisture free or active to dissolve the salts, while in the second case there is only 7 per cent of free or active water for the same purpose. The depression of the freezing point at the low moisture content therefore would be many times greater than that at the high than would be expected from the total percentage of water content.

It was also found that the magnitude of the lowering of the freezing point of soils at the low moisture content decreased with successive freezings. This was true, however, only with complex and colloidal types of soil, such as the silts, loams, and clays, and not with the simple and noncolloidal soils, such as the sands and light sandy loams.

In explanation of this phenomenon the hypothesis was offered that a large portion of the water which was made inactive or unfree and thus removed from the field of action as far as the freezing-point lowering is concerned, was due to the colloids which the soils contain. This inactive or unfree water existed in the colloids both as physically adsorbed and loosely chemically combined water. Upon freezing, these colloids coagulated, the bonds uniting them with the water broke, and the combined or unfree water became liberated. This liberated and free water went to dilute the original solution and thus decreased the lowering of the freezing point. Thus, for instance, if there were 5 per cent of moisture at the beginning of the first freezing, there were probably 7 per cent at the end of the first freezing, 7.5 per cent at the end of the second freezing, and so on, until all the colloids were coagulated.

It was further discovered in these researches that solidification could not be induced to take place below a certain minimum moisture content, but it could be started extremely easily above this critical point. This minimum water content varied with the type of soil and appeared to correspond quite closely to the moisture content at which plants begin to wilt, or the wilting coefficient of soils. Since the percentage of moisture in many of the soils was quite high, amounting in some clays to 20 per cent and in some humus clays to 40 per cent and yet solidification could not be started, it appeared logical to conclude that the remaining water in the soil did not exist in a free state but in an inactive or unfree condition.

All evidences therefore seemed overwhelmingly in favor of the view that soils cause some of their moisture to become inactive or unfree and thus lose its solvent action and be removed from the field of action, so far as the freezing-point lowering is concerned.

The desire now arose to measure quantitatively the amount of this water which the different soils cause to become inactive or unfree and lose its solvent action. Such a determination, it was thought, would be of considerable scientific and practical importance.

After a thorough consideration it became apparent that the freezing-point method could not give a quantitative estimation of this inactive or unfree water without having to follow a rather complicated procedure. It could show only qualitatively that soils do cause water to become inactive and that the amount of this inactive water varied with the different soils. A search therefore was instituted to find some method which was simple, rapid, and accurate, and which could determine quantitatively the amount of water that the various soils cause to become inactive. After a considerable search the dilatometer method was chosen as the most promising for accomplishing the object.

The dilatometer method is an old one. The writer used it in the physical-chemical laboratory of Prof. G. Tamman at Göttingen University in 1913. Its employment, however, in the present study received the impetus from the work of Foote and Saxton (5), of Yale University.

It is an interesting coincidence that this work of Foote and Saxton, which really deals with the different forms of water held by the various hydrogels, appeared in March, 1916, while the writer's work (2), which contains the evidences bearing upon the ability of soils to cause water to become inactive, appeared in December, 1915, or only three months earlier. It will be of interest to mention that Foote and Saxton, using the dilatometer method, and the writer the freezing-point method, arrived independently at the conclusion that a considerable amount of water in certain solid substances refused to freeze, and consequently must exist in an inactive or combined form.

Although the idea of employing the dilatometer method to attain the writer's object received its impetus from the work of Foote and Saxton, the procedure followed and the form of apparatus used were devised in the course of the investigation and are entirely original and very different. As a matter of fact, Foote and Saxton give no description of the apparatus they employed, merely saying, "The dilatometer had the usual form."

PRINCIPLE OF THE METHOD

The principle of the dilatometer method as employed in the present investigation is based upon the fact that water expands upon freezing. If the amount of expansion that a certain quantity of water, 1 gm., produces upon freezing is known, then the amount of water that freezes in the soil can be calculated from the magnitude of expansion produced. If, also, the total water content in the soil is known, the amount that does not freeze can be ascertained by the difference.

DESCRIPTION OF THE METHOD AND PROCEDURE

A large amount of time was spent in endeavoring to devise a dilatometer which would be simple and give accurate and concordant results. A large number of dilatometers of different forms were tried; but they were

all discarded, and the one shown in figure 1 was finally adopted and employed throughout this investigation. It consists of a glass bulb, A, in which the soil is placed, the glass stem, B, on which the expansion is read, and the thermometer, C, which serves to indicate the temperature of the soil or mass and also acts as a stopcock to the bulb A. The bulb has a capacity of 50 c. c.; the stem is a 1 c. c. pipette and is calibrated to 0.1 c. c.; and the thermometer reads from $-5°$ C. to $+30°$, and is calibrated to 1°. That portion of the thermometer which is inserted into the mouth of the bulb is suitably ground down so that it forms water-tight connections with the latter.

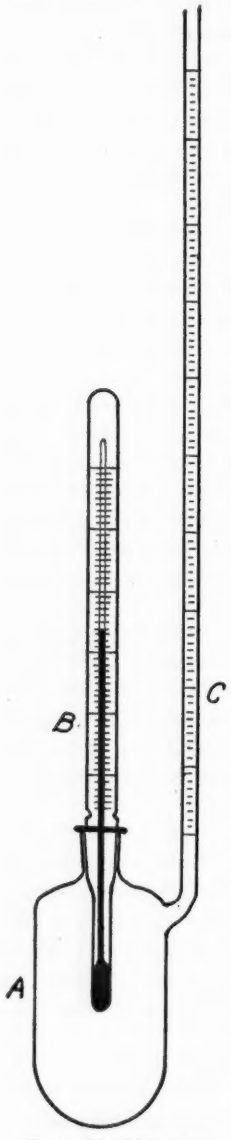

FIG. 1.—The dilatometer

It was found absolutely necessary to have a thermometer in the dilatometer, in order to indicate the degree of supercooling, because the amount of water that freezes in the soil is influenced to a considerable extent by the degree of supercooling. If the magnitude of supercooling was not the same, no concordant results could be obtained in the different samples of the same kind of soil.

For completely filling the bulb of the dilatometer after the soil was added and for measuring the volume of expansion of the soil water upon freezing, ligroin was used. Ligroin, of course, is a derivative of petroleum and is sometimes called "light petroleum." It proved to be a very satisfactory material for completely filling the bulb and for measuring expansion.

For determining the amount of water that soils cause to become inactive or unfree, the following procedure was adopted: To 25 gm. of air-dry soil were added 5 c. c. of distilled water. These were thoroughly mixed. In those soils which possessed a high water-holding capacity the mixing was done in an evaporating dish before the soil sample was placed in the dilatometer, while in those soils which possessed a low water-holding capacity the soil sample was first placed in the dilatometer and then the water added to it. In both of these processes precaution was taken to guard against the loss of water through evaporation and thus to avoid inequalities.

After the soil was placed in the bulb, the latter was completely filled with the ligroin. For expelling the air the soil was stirred with a rod until bubbles ceased to appear at the top. The process of stirring proved quite efficient in facilitating the removal of air.

After all the air was expelled from the soil, the thermometer was inserted into the mouth of the dilatometer and a sufficient amount of ligroin was placed in the stem, so that when the mass in the bulb had cooled, the column of the ligroin would be at the lower part of the graduated portion of the stem. The stem was then covered by a special cape in order to prevent the loss of the ligroin through volatilization.

The bulb of the dilatometer was then placed in a cooling mixture and allowed to cool. The cooling mixture consisted of crushed ice and common salt and was contained in a bath composed of two earthenware jars placed one inside the other and well insulated. The temperature of the cooling mixture was maintained at $-4°$ C. The dilatometer was allowed to remain undisturbed until the contents had attained the temperature of $-3°$, that is, supercooled to $3°$; and then by taking hold of the dilatometer by the stem it was gently moved in the cooling mixture until solidification began. The beginning of the solidification was indicated by the rise of the ligroin in the stem. The bulb of the dilatometer was allowed to remain in the cooling mixture, with a frequent movement until the rise of the liquid in the stem ceased. The time required for equilibrium to be attained varied with the type of soil, but it generally took about half an hour. This length of time, however, could be considerably reduced by moving the bulb in the ice mixture very often. The total rise of the ligroin in the stem was taken to represent the total amount of expansion due to the formation of ice.

Whenever it was desired to study the effect of successive freezings upon the amount of water that freezes, the bulb of the dilatometer was taken out of the ice mixture, wiped dry, and held between the hands until the soil was thawed, as indicated by the rise of the temperature on the thermometer; then it was placed back into the cooling mixture and the process described above was repeated.

For obtaining concordant results it was found essential to supercool the soil to the same degree. As will be shown subsequently, the amount of water that will freeze in a soil increases with the increase in supercooling. This is especially true of colloidal soils.

It was not found practicable to maintain in the bath a lower temperature below zero than $-4°$ C. If the temperature was lower than $-4°$, solidification would start before supercooling had taken place and no satisfactory results could be obtained on expansion. Furthermore, if the bath was too cold, the tendency for contraction would be too great, and the total expansion might not be indicated in the stem.

In performing tests to ascertain how closely duplicate determinations would agree by following the same procedure as closely as possible, it was found that in the majority of cases the duplicate determinations of the same kind of soil would agree very closely. Occasionally, however, the disagreement would be quite appreciable. This disagreement may be attributed partly to three factors.

In the first place, it appears that the phenomenon of solidification or crystallization does not start always the same; sometimes it starts with difficulty and its velocity is small, while at other times it starts very readily and its velocity is very high, even though the amount of supercooling in both cases is the same.

In the second place, the force of expansion may not be evenly distributed throughout the bulb, and consequently the entire expansion may not be indicated in the stem. It is perhaps due to this factor that stirring or moving the bulb in the cooling mixture hastens the equilibrium. This equilibrium would doubtless be attained in a shorter time, and the results would be much more accurate if the stirring was performed in the bulb. A dilatometer was devised in which the stirring could be performed in this manner. This dilatometer consisted of a long tube having a ground joint, and on this joint were attached the thermometer and the measuring stem. The contents of the tube were stirred by an electrical arrangement similar to that employed in the Beckman apparatus for performing freezing-point-lowering determinations. Unfortunately tubes with ground joints such as were necessary could not be obtained now in this country; and since it was necessary to have several of them, this form of dilatometer had to be abandoned.

In the third place, the water which the soils cause to become inactive or unfree is not in an absolute unchangeable condition, but is made free by various factors. Hence, if the empirical procedure of the method is not exactly the same, the duplicate determinations would naturally not agree.

In order to obtain the correct factor or value for converting the expansion due to ice formation into the corresponding weight, the expansion of 5 c. c. of distilled water was determined in the dilatometer employed. It was found that these 5 c. c. of water gave an expansion of about 0.5 c. c. upon freezing. According to these figures, 1 c. c. of water expands to about 0.1 c. c. upon freezing. This value is somewhat higher than that obtained by other investigators. Bunsen's data, for instance, show that 1 gm. of water at 0° C. expands 0.09070 c. c. upon freezing. In the computation of the data presented in this paper, however, the former value was employed. It is believed that for the writer's purpose this value is sufficiently correct; it is also very convenient.

EXPERIMENTAL WORK

QUANTITY OF WATER SOILS CAUSE TO BECOME UNFREE, AS INDICATED BY QUANTITY OF WATER THAT FAILS TO FREEZE

In accordance with the foregoing method, the amount of water that the soils cause to become unfree or inactive as indicated by the quantity of water that fails to freeze was determined. The soils employed varied both in type and origin. As to origin, many of the soils used had come from several different States. The results obtained are shown in Table I. The amount of water that fails to freeze is expressed both in cubic centimeters and in percentage of the 5 c. c. of water added to 25 gm. of soil. In every case the temperature of the bath was $-4°$ C., the soil was supercooled to 3°, and the readings are only of the first freezing.

TABLE I.—*Amount of water that fails to freeze in 5 c. c. added to different soils*

State and name of soil.	Water failing to freeze.	Added water failing to freeze.	State and name of soil.	Water failing to freeze.	Added water failing to freeze.
	C. c.	*Per cent.*		*C. c.*	*Per cent.*
Quartz sand	0.10	2.0	Florida:		
Michigan:			Sand	0.50	10.0
Sand	.30	6.0	Do	.50	10.0
Sandy loam	.80	16.0	Do	.50	10.0
Do	1.50	30.0	Do	.55	11.0
Do	1.15	30.0	Minnesota:		
Silt loam	2.00	40.0	Sand	.50	10.0
Do	2.30	46.0	Sandy loam	1.50	30.0
Heavy silt loam	2.80	56.0	Clay	4.00	80.0
Do	3.10	62.0	Washington:		
Do	3.50	70.0	Sand	.50	10.0
Do	3.70	74.0	Fine sandy loam	1.15	23.0
Kentucky:			Silt loam	1.65	33.0
Sandy loam	.90	18.0	Do	2.00	40.0
La Crosse sandy loam	1.15	23.0	Do	2.00	40.0
Parrington loam	2.80	56.0	Heavy silt loam	2.20	44.0
Marshall silt loam	2.90	58.0	Wisconsin:		
Texas:			Plainfield sand	.40	8.0
Sand	.20	5.0	Miami silt loam	1.40	28.0
Do	.30	6.0	Carrington silt loam	1.00	32.0
Fine sandy loam	.50	10.0	Colby silt loam	2.20	44.0
Sandy loam	.90	18.0	Superior clay	3.50	70.0
Crawford clay	2.70	54.0	Pennsylvania:		
Houston black clay	3.00	60.0	Silt loam	1.40	28.0
			Do	1.50	30.0
			California:		
			Clay loam	2.00	40.0

The results presented in Table I are really of great significance. They show that not all of the water added to the soils freezes; some of it refuses to freeze, and the amount that fails to freeze is entirely different in the various soils. It varies from 2 per cent in the case of quartz sand to 80 per cent in the case of Minnesota clay, of the 5 c. c. of water added to 25 gm. of soils. It increases, therefore, from the simple and noncolloidal types of soil to the complex and colloidal types.

If it is assumed that only free water freezes, then the water which fails to freeze is inactive or unfree, and has been transformed into this condition by the soils themselves.

RELATION BETWEEN THE AMOUNT OF WATER THAT FAILS TO FREEZE AND THE LOWERING OF THE FREEZING POINT

The amount of water that fails to freeze under the above empirical conditions bears a significant relation to the lowering of the freezing point. In Table II there are presented the percentage of water that fails to freeze and the value of the freezing-point lowering of a few typical soils. The depression was obtained by mixing 20 gm. of soil with 4 c. c. of water, thus making a ratio of 5 of soil to 1 of water. This ratio is exactly the same as that employed in the dilatometer determinations.

TABLE II.—*Amount of water that fails to freeze in 5 c. c. added to various soils and their corresponding lowering of the freezing point*

Name of soil.	Water failing to freeze.	Added water failing to freeze.	Lowering of the freezing point.
	C. c.	*Per cent.*	*°C.*
Quartz sand	0.1	2.0	0.009
Sand	.3	6.0	.074
Do	.4	8.0	.019
Do	.5	10.0	.018
Do	.5	10.0	.017
Do	.5	10.0	.011
Sandy loam	1.7	34.0	.138
Silt loam	2.0	40.0	.200
Superior clay	3.5	70.0	.392
Heavy silt loam	3.5	70.0	.312
Do	3.7	74.0	.440
Houston black clay	3.0	60.0	.422

An examination of the data of Table II reveals at once the significant fact that there is an exceedingly close relation between the amount of water that fails to freeze in the various soils and the degree of their freezing-point lowering. Those soils, for instance, which show the greatest lowering of the freezing point allowed the smallest percentage of water added to freeze, while those soils which indicate the smallest freezing-point depression permitted the largest percentage of water added to freeze.

The fact that not all of the added water freezes and the remarkable correlation between the percentage of unfrozen water and the lowering of the freezing point bear out the hypothesis proposed in former publications (2) that the abnormally high depression of soils at the low moisture content, or the abnormally great increase of the depression with the decrease in moisture content, is due to the ability of soils to cause water to become inactive or unfree and thus be removed from the field of action so far as the freezing-point lowering is concerned.

RELATION BETWEEN THE AMOUNT OF WATER THAT FAILS TO FREEZE AND THE WILTING COEFFICIENT OF SOILS

The amount of water that fails to freeze under the foregoing empirical conditions bears also a remarkable relation to the wilting coefficient of soils. In Table III there are presented the total percentage of water that fails to freeze and the wilting coefficient of some soils. The percentage of water that fails to freeze is expressed on the absolute dry basis, in order that it may be directly comparable to the wilting coefficient which is similarly expressed. The data on the wilting coefficient were obtained experimentally, following the mode of procedure described by Briggs and Shantz (4). Wheat was used as an indicator.

TABLE III.—*Relation between the percentage of water that fails to freeze and the wilting coefficient*

Name of soil.	Percentage of water that fails to freeze.	Wilting coefficient.
Sand	1.40	1.49
Sandy loam	3.52	4.28
Silt loam	10.50	9.62
Heavy silt loam	17.30	18.16
Do	15.86	13.82

A glance at Table III discloses at once the remarkable fact that the total percentage of water which does not freeze when the soil is supercooled to 3° in a temperature of −4° and the ratio of soil to water is 5 to 1, respectively, is the same as that at which plants begin to wilt, or the wilting coefficient. It will be observed that the variation does not exceed 2 per cent in any case.

RELATION BETWEEN THE AMOUNT OF WATER THAT FAILS TO FREEZE AND THE PERCENTAGE OF WATER AT WHICH SOLIDIFICATION REFUSES TO TAKE PLACE

As already mentioned, the investigations with the freezing-point method showed that solidification could be induced in all soils very easily from any maximum moisture content down to a minimum moisture content, and then it could not be started. It was shown in a former publication (2) that the percentage of water at which solidification could not be induced was the same as the wilting coefficient of soils, and it was suggested that the freezing-point method may be used to obtain the latter value. It may now be of interest to indicate that the percentage of water at which solidification refuses to start bears a close relation also to the amount of water which refuses to freeze in the dilatometer. This interesting correlation is exhibited in Table IV.

TABLE IV.—*Percentage of water that fails to freeze and the percentage of water content at which solidification refuses to take place*

Name of soil.	Percentage of water at which solidification refuses to take place.	Percentage of water that fails to freeze.
Sand	2. 10	1. 40
Sandy loam	5. 60	3. 52
Silt loam	10. 30	10. 50
Heavy silt loam	16. 00	17. 30
Heavy silt loam	13. 50	15. 86

It becomes at once evident from an examination of the foregoing results that the percentage of water at which solidification can not be induced to start and the percentage of water which fails to freeze are quite close in all the different types of soil.

RELATION BETWEEN THE AMOUNT OF WATER THAT FAILS TO FREEZE AND THE THERMAL CRITICAL MOISTURE CONTENT

In investigating the effect of temperature on some of the most important physical processes in soils, the effect of temperature on the movement of water vapor and capillary moisture was also studied (3). It was found that when one-half of a column of soil of uniform moisture content was maintained at 20° and 40° C. and the other half at 0° for eight hours, the percentage of water moved from the warm to the cold soil increased in all the different types of soil with the rise in moisture content until a certain water content was reached and then it decreased with further increase in the moisture content. The results then plotted into a parabola. The percentage of moisture at which the maximum thermal translocation of water occurred was different in the various classes of soil, but the percentage of the maximum thermal translocation of water was about the same for all classes for any one of the temperature amplitudes employed. The percentage of moisture at which this maximum translocation occurred was designated as "thermal critical moisture content."

It is now of great interest to state that this thermal critical moisture content is practically the same as the percentage of water which refuses to freeze in the corresponding soils—that is to say, the percentage of water in the soil at which the maximum thermal translocation occurs is practically the same as the percentage of water which refuses to freeze. This significant correlation was tested in about 10 different soils, and in almost every case the relation was exceedingly close, the variation not exceeding more than 3 per cent water content in any soil.

SIGNIFICANCE OF THE AGREEMENT OF THE VARIOUS METHODS IN INDICATING THE SAME PERCENTAGE OF WATER AS THAT WHICH FAILS TO FREEZE

The agreement of the various methods of entirely different principle in giving practically the same percentage of moisture as that which fails to freeze under the empirical conditions already mentioned is of great significance. This remarkable agreement indicates that there is a critical point on the moisture curve or scale at which the condition of the soil moisture begins to change, and this change or transformation must be very marked. At this point the soil water appears to change from the free state to an inactive or unfree condition. Whether or not the point of transition is abrupt can not be said definitely, but many evidences indicate that it is quite sudden.

On the other hand, it must be stated that these various methods probably do not indicate the absolute initial point of commencement of the unfree water. It is almost certain that the degree of unfree water as shown by the various methods is somewhat below that of the total. This is due to the fact that the unfree water does not exist in an absolute unchangeable and unutilizable condition, but in a state in which it can be changed into free water by various factors. As a consequence, the methods are not absolute, but are empirical—that is to say, since this unfree water can be converted into free water by various factors, then the amount of unfree water that any methods indicate will depend to a considerable extent upon the empirical conditions employed in that method. Thus, for instance, as will be shown subsequently, the amount of water that fails to freeze decreases with the increase in supercooling; the amount of unfree water therefore diminishes with greater undercooling. Again, the wilting coefficient of soils is not absolutely definite, but varies to a considerable extent with the environmental conditions which affect the intensity or velocity of atmospheric evaporation.

The agreement of the foregoing methods, therefore, in indicating the same percentage of unfree water must be considered more as a remarkable coincidence rather than as indicating the absolute initial point of commencement of the unfree water and consequently the absolute total amount of this unfree water.

EFFECT OF SUCCESSIVE FREEZINGS UPON THE AMOUNT OF WATER THAT FAILS TO FREEZE IN SOILS

The work with the freezing-point method showed, as already mentioned, that the lowering of the freezing point of colloidal soils at low moisture content decreased with successive freezings up to a certain number of freezings and then it became constant. This diminution was interpreted to mean that the soils had caused some of the water to

become inactive or unfree, to lose its solvent action, and to be removed from the field of action so far as the freezing-point lowering is concerned, and that the process of freezing changed the physical condition of the soil whereby some of this inactive or unfree water was liberated and went to dilute the original soil solution and thus decreased the lowering of the freezing point.

In order to ascertain whether this hypothesis was correct, the effect of successive freezings upon the amount of water that would freeze in soils was studied in the dilatometer. The procedure consisted of refreezing the same sample of soil in the dilatometer as was employed in the previous study. After the data of the first freezing were obtained, the dilatometer was taken out of the bath, its contents thawed, and the process of freezing was repeated a second time. Numerous trials showed that in the majority of cases equilibrium was attained in the second freezing—that is, very little, if any, more water froze after the second freezing. Many trials also showed that not very much more water would freeze if the soil was maintained at a temperature of $-12°$ C. for one hour after the first freezing, than by supercooling it to $3°$ at a temperature of $-4°$, as followed in the regular procedure. In all the determinations made in this study, therefore, the soil was refrozen a second time in the same manner as in the first—that is, supercooled to $3°$ in a cooling mixture of $-4°$. The data obtained are detailed in Table V. The amount of water that fails to freeze in the first and second freezings is expressed both in cubic centimeters and in percentage of the 5 c. c. of water added to 25 gm. of soil. The difference in the percentage of water that fails to freeze in the first and second freezings indicates the effect of successive freezings upon the amount of water that becomes free.

TABLE V.—*Effect of successive freezings upon the amount of water that fails to freeze when 5 c.c. are added to various soils*

Freezing period.	Water in quartz sand failing to freeze.		Water in sand failing to freeze.		Water in sandy loam failing to freeze.				Water in silt loam failing to freeze.		Water in Carrington loam failing to freeze.		Water in heavy silt loam failing to freeze.				Water in superior clay failing to freeze.	
					First test.		Second test.						First test.		Second test.			
	C.c.	%	*C.c.*	%	*C.c.*	%	*C.c.*	%	*C.c.*	%	*C.c.*	%	*C.c.*	%	*C.c.*	%	*C.c.*	%
First	0.1	2.0	0.3	6.0	0.8	16.0	1.15	23.0	2.00	40.0	2.80	56.0	3.70	74.0	3.50	70.0	3.50	70.0
Second	.1	2.0	.3	6.0	.8	16.0	1.15	23.0	1.65	33.0	2.20	44.0	2.75	55.0	3.05	61.0	3.00	60.0
Difference	.0	.0	.0	.0	.0	.0	.0	.0	.35	7.0	.60	12.0	.95	19.0	.45	9.0	.50	10.0

The foregoing data show that the amount of water which fails to freeze in the second freezing is practically the same as in the first in the case of sands and light sandy loams or noncolloidal soils, but in the silts, loams, clay loams, and clays, or complex and colloidal types it is considerably less in the second freezing than in the first. In other words, repeated freezings increase the amount of free water and decrease to a corresponding degree the quantity of unfree water in the case of complex and colloidal types of soil but not in the simple and noncolloidal types of soil. As will be observed, in some of the loams and clays about 19 per cent more water froze in the second than in the first freezing.

These results therefore demonstrate that successive freezings cause some of the unfree or inactive water to become free in some soils and not in others. They go to prove, therefore, the validity of the hypothesis already mentioned, that the diminution of the lowering of the freezing point of colloidal soils at low moisture content with successive freezings is due to the dilution of the initial soil solution brought about by the inactive water in the soil becoming free through the process of freezing.

It must have been already realized, however, that it is not only the freezing that causes the unfree or inactive water to become free but also the thawing. If it were due only to freezing, then all the water that would become free would have done so during the first freezing. Instead, a large amount of water becomes free after the soil is thawed and frozen a second time.

Finally, it must be stated that if 10 c. c. of water are added to the 25 gm. of soil instead of 5 c. c., as was done above, the amount of water that fails to freeze in the second freezing is practically the same as that in the first, even in the complex colloidal types of soil. These results confirm those obtained with the freezing-point method, which show that the degree of the lowering of the freezing point of all types of soil at very high moisture content remains quite constant with successive freezings.

EFFECT OF DEGREE OF SUPERCOOLING UPON THE AMOUNT OF WATER THAT FAILS TO FREEZE

The amount of water that fails to freeze is dependent to a considerable extent upon the degree of supercooling. This is, however, true only in the complex and colloidal soils and not in the simple noncolloidal soils. The data bearing upon this phase of the investigation are presented in Table VI. These data were obtained at two degrees of supercooling, 1° and 3° C., in a cooling mixture of −4°. For each degree of supercooling a new sample of soil was used.

From an inspection of the data in Table VI it becomes at once clearly evident that the amount of water that fails to freeze is considerably less when the soil is supercooled to 3° C. than when it is supercooled to 1°. This, however, is true only in the case of the complex types of soil, the silts, loams, clay loams, and clays, but not in the simple types of soil,

such as the quartz sand, sands, and sandy loams. In some of the clay loams and clays the amount of water that fails to freeze in the supercooling of 3° is about 18 per cent less than in the supercooling of 1°.

TABLE VI.—*Effect of supercooling upon the amount of water that fails to freeze when 5 c.c. are added to various soils*

Degree of supercooling.	Water in quartz sand failing to freeze.		Water in sand failing to freeze.		Water in silt loam failing to freeze.		Water in heavy silt loam failing to freeze.					
							First test.		Second test.		Third test.	
	C.c.	*P. ct.*	*C.c.*	*P. ct.*	*C.c.*	*P. ct.*	*C.c.*	*P. ct.*	*C.c.*	*P. ct.*	*C.c.*	*P. ct.*
To 1° C.	0.1	2.0	0.4	8.0	3.10	62.0	4.15	83.0	3.70	74.0	3.30	66.0
To 3° C.	0.1	2.0	0.3	6.0	2.20	44.0	3.50	70.0	3.10	62.0	2.80	56.0

Attempts were made to obtain data at still greater supercoolings than 3° C.; but it was found that if the temperature of the bath was increased above −4° in order to obtain the greater supercoolings, the soil began to freeze when it was cooled only a few tenths of a degree below zero. The highest temperature that could be obtained at which premature solidification would not start was −4°. Hence, the greatest supercooling that was possible at this temperature was 4°. For rapidity of operation, however, the supercooling of 3° was employed.

The decrease in the amount of the water that fails to freeze in the colloidal soils with the increase of supercooling is highly significant. It indicates that supercooling lends a certain amount of energy to the force of crystallization which overcomes the forces of the soils that cause the water to become unfree, and liberates this water so it can freeze. This decrease in the amount of water that fails to freeze with an increase in the supercooling bears an analogy to the increased quantity of the solid solvent that will separate from the solution with the increase in the degree of supercooling. It is a well-known fact that the amount of pure solvent separating into its solid phase upon freezing is increased with the degree of supercooling, and thus concentrates the remaining solution.

The above results also go to bear out the statement already made that the unfree water does not exist in the soil in an unchangeable condition, but that it can be made free by various factors, and that the magnitude depends upon the empirical condition of the method employed for its determination.

EFFECT OF THE AMOUNT OF MOISTURE PRESENT UPON THE AMOUNT OF WATER THAT FAILS TO FREEZE

In the course of the development of the procedures of the dilatometer method it was noticed that the quantity of moisture present in the soil influenced somewhat the amount of water that failed to freeze. In order to obtain very definite data upon this subject a series of determinations was performed with soils at two different water contents, 5 and 10 c. c. of water in 25 gm. of soil. The data obtained are detailed in Table VII.

TABLE VII.—*Effect of degree of water content present upon the amount of water that fails to freeze*

Name of soil.	Quantity of water failing to freeze when 5 c. c. of water were added.	Quantity of water failing to freeze when 10 c. c. of water were added.
	C. c.	*C. c.*
Quartz sand	0. 10	0. 10
Sand	.30	.40
Sandy loam	1. 15	1. 00
Silt loam	2. 00	1. 60
Heavy silt loam	3. 10	2. 30
Do	3. 70	2. 50
Do	3. 50	2. 60
Do	2. 80	2. 20
Clay	4. 00	2. 80

There are two columns of data in Table VII, one showing the amount of water that failed to freeze when 5 c. c. of water were added to 25 gm. of soil and the other when 10 c. c. of water were added to the same quantity of soil. An examination of the results in these two columns shows at once that actually a greater amount of water failed to freeze when 5 c. c. of water were added than when 10 c. c. were added. This is true, however, only in the case of the complex colloidal types of soil and not in the simple noncolloidal types. Thus, in the case of one of the heavy silt loams the amount of water that failed to freeze when 5 c. c. were added is 3.5 c. c., while the amount that failed to freeze when 10 c. c. were added is 2.6, or a difference of 0.9 c. c. It will be observed that in almost every soil of the complex colloidal classes, about 1 c. c. more water failed to freeze when 5 c. c. were added than when 10 c. c. were added. In the case of the simple noncolloidal classes of soil, such as the sands, that quantity of water that failed to freeze was the same in both the smaller and larger quantity of water added.

The foregoing data in the case of the colloidal soils are really very significant. They indicate that the absolute amount of inactive or unfree water is greater at low moisture content than at high. Evidently an excess of water diminishes the quantity of water that the colloidal soils cause to become inactive or unfree.

Just why the quantity of water that fails to freeze in the colloidal soils is greater at the low moisture content than at the high can not be explained at this time definitely. The following suggestion, however, may be offered: It is probably partly due to the fact that the total force of solidification is greater at the higher moisture content than at the lower, and consequently more water is freed or extracted from the soil in the former than in the latter. In short, the rôle of the relative masses present comes into play.

CONDITION OF THE UNFREE WATER IN THE SOILS

It has been seen, therefore, that there is a certain amount of water in soils which refuses to freeze, the exact amount varying with the degree of supercooling, number of times the soil is frozen, and degree of moisture present. Under certain empirical conditions—25 gm. of air-dry soil mixed with 5 c. c. of water supercooled to 3° C. in a temperature of −4°—the percentage of water, on the absolute dry basis, that remains unfrozen corresponds remarkably closely to the moisture content known as the wilting coefficient, to the water content at which solidification can not be induced, etc. In order to distinguish this water from the free water which freezes easily, is readily available to plants, etc., it is designated as "unfree" water.

The question now is, How does this unfree water exist in the soil? Does it exist as capillary water, physically adsorbed water, loosely chemically combined water, or in all forms?

It must be stated in advance that it is not definitely known in which form or forms this unfree water exists. The problem is very complex and exceedingly difficult of definite solution. It is, however, under special investigation, and it is hoped that a definite solution of it may be presented at a later date. However, it may be stated at this time that there are many evidences, both direct and indirect, which indicate quite strongly that most of this unfree water exists as physically adsorbed and loosely chemically combined, with the latter probably predominating. Capillary water, as ordinarily understood, is present in small quantities. It probably abounds mainly in the initial region of the unfree water. The physically adsorbed and loosely chemically combined water probably exists in the solid phase or as solid solution.

The following are some of the evidences which go to indicate that most of the unfree water exists in a physically adsorbed and loosely chemically combined condition and consequently in the solid phase or as solid solution:

(1) As it has already been stated, the studies on the lowering of the freezing point of soils showed that this value increased in all soils with the exception of quartz sand and some extreme types of sand at a far greater rate than the percentage of water decreased. In other words, the ratio of the freezing-point lowering and the percentage of water were not inversely proportional (approximately) as might be expected, save only in the quartz sand and some of the sands. Thus, a clay at 92.76 per cent of moisture gave a depression of 0.039° C. and at 39.28 per cent, 1.075; the ratio of the percentage of water at the two moisture contents is only 2.37, while that of the depression is 27.56.

This phenomenon was explained on the supposition that some of the water contained by the soils was either physically adsorbed or loosely chemically combined or both, in which event this portion of the water

was not free or active to act as a solvent but was removed from the liquid phase and consequently also from the field of action, so far as the freezing-point lowering is concerned. Under this assumption the results could be easily explained. Thus, for example, if a clay caused 15 per cent of water to become inactive or unfree, either physically or chemically, or both, and at 39 per cent of moisture this clay gave a depression of 0.075° C., and at 22 per cent, 0.987°, then in the first case there was 24 per cent of free and active water to dissolve the salts and take part in the freezing-point lowering, while in the second case there was only 7 per cent of free and active water for the same purpose. The depression of the freezing point at the low moisture content therefore would be many times greater than that at the high, than would be expected from the total percentage of moisture content.

(2) It was also found in these researches on the freezing-point lowering of soils that the magnitude of the depression decreased with successive freezings. This was true, however, only in the complex and colloidal types of soil such as the silts, loams, clay loams, and clays, and not in the simple and noncolloidal types such as the quartz sand, sands, and light sandy loams.

In explanation of this phenomenon the hypothesis was offered that a large portion of water which was made unfree or inactive and thus removed from the liquid phase was due to the colloids which the soils contained. This unfree or inactive water existed in the colloids both as physically adsorbed and loosely chemically combined. Upon freezing, these colloids coagulated and the bonds uniting them with the water broke and the combined or unfree water became liberated and free. This liberated and free water went to dilute the original soil solution and thus decreased the lowering of the freezing point. Thus, for instance, if there were 5 per cent of free water at the beginning of the first freezing, there were probably 7 per cent at the end of the first freezing, 7.5 per cent at the end of the second freezing, and so on until all the colloids were coagulated.

It might appear that the foregoing data do not prove that the unfree water exists as physically adsorbed and loosely chemically combined and probably in the solid phase. It could also exist as capillary and film water in the liquid phase. If it is in the liquid phase, why does it not take part in dissolving the salts like the free water does, and be in equilibrium with the latter, so that the freezing-point lowering would not follow such an abnormal course?

(3) The view that some of the unfree water in the soil may exist as physically adsorbed and loosely chemically combined probably in the solid phase is considerably strengthened by the results obtained by Jones (6, p. 238) on the freezing-point lowering of hydrates. Jones found that the different hydrates such as calcium chlorid, magnesium

bromid, aluminium chlorid, etc., yielded far greater depression of the freezing point than would be theoretically expected. Jones attempted to explain these abnormal results by assuming that these hydrates take up water, forming complex compounds with it, and thus remove it from the field of action, so far as the freezing-point lowering is concerned.

(4) The chemical composition of the soil itself would probably seem to presuppose that some of the water in the soil must be chemically combined, if some of our prevalent chemical knowledge is correct. The present status of our knowledge of the chemical composition of soils indicates that the soil contains many colloidal hydrates, such as aluminium, silica, iron, and magnesium, in simple or in complex combinations or zeolites. If these compounds exist in the hydrate form, then the water of hydration, according to our present conception, is probably chemically combined.[1] The amount of water that many of these hydrates take up and combine with it, probably chemically, is really very great, as will be seen from the following formulæ: $Al_2O_3 \cdot 38H_2O$, $SiO_2 \cdot 1.35H_2O$, $Fe_2O_3 \cdot 4.25H_2O$, $Na_2SO_4 \cdot 10H_2O$, $Na_2CO_3 \cdot 10H_2O$, $CaCl_2 \cdot 6H_2O$, etc. The force with which this water of hydration is held by the different hydrates varies considerably. Such hydrates as $Na_2SO_4 \cdot 10H_2O$ lose most of their water upon being exposed to the ordinary atmosphere, while others, such as $CaCl_2 \cdot 6H_2O$, will lose it only at elevated temperature. Furthermore, all the water in any hydrate is not held with the same force but with a different degree of force. This is shown by the fact that the same hydrate possesses different degrees of aqueous pressure at the various degrees of hydration or formula weights of water. Thus, copper sulphate in the form of $CuSO_4 \cdot 5H_2O$ exhibits an aqueous pressure of 47 mm., while in the form of $CuSO_2 \cdot H_2O$ it shows an aqueous pressure of only 4.5 mm., at a temperature of 50° C. That all the water in a hydrate is not held by the same force is further confirmed by experimental data, which show that the greatest portion of water of hydration of most hydrates is lost below the temperature of 100°, while the remainder is given off above this temperature.

(5) That some of the unfree water may exist in the soil as chemically combined is further indicated by the researches of Muntz and Gandechon (8) on the heat generated by dry soils upon being wetted. These investigators found that a large amount of heat was generated when the soils were wetted with water, but very little if any when they were brought in contact with benzene and toluene. They reasoned that, if the heat

[1] The vapor tension of the salt hydrates, such as the $CaCl_2 \cdot 6H_2O$, $Na_2SO_4 \cdot 10H_2O$, etc., behaves quite different from that of the colloidal hydrates, such as the $Al_2O_3 \cdot 38H_2O$, $SiO_2 \cdot 1.35H_2O$, etc. In the former hydrates the vapor tension decreases by sudden steps as water is being withdrawn, indicating that definite hydrates are being formed. In the latter hydrates, however, the vapor tension decreases continuously without any sudden break in the curve, indicating probably that no definite hydrates are being formed. As a result of this difference in the vapor tension of the two classes of hydrates some investigators are led to believe that the water in the colloidal hydrates exists as physically adsorbed water and not as loosely chemically combined water. The opposite view is held by other investigators. Apparently the subject has not been definitely solved one way or the other. The problem is exceedingly difficult of definite solution.

generated is due to physical phenomena only, then the amount of heat produced must have been the same in the benzene and toluene as that in the water. They also found that soils would extract the water from alcohol. From all these data they concluded, although not positively, that water must be chemically combined in soils.

(6) In the present investigation it was found that if those soils which cause a large amount of water to become unfree, as indicated by the quantity that fails to freeze, are heated to red heat, they cause then very little if any water to become unfree. Indeed, they act almost like quartz sand. Their power to render water inactive, therefore, is destroyed by the process of heating.

These results could be interpreted to mean that these soils contain colloids, that it is these colloids which render the water inactive by combining with it physically and chemically to form colloidal hydrates, and that these colloids are destroyed by heating and are converted into an irreversible condition so that they can not take up the water to combine with it physically and chemically and assume the hydrated state again.

All the foregoing evidences therefore appear to indicate quite strongly that some of the unfree water exists in the soils as physically adsorbed and chemically combined.

In view of the presence of the colloidal hydrates in the soils and in view of the tremendous amounts of water that many of these colloidal hydrates take up, it also appears that the greater portion of the unfree water in the soils and especially in the colloidal types is chemically combined rather than physically adsorbed, provided the water in the colloidal hydrates of alumina, silica, and iron exists as loosely chemically combined water and not as adsorbed water.

The question now is, if this chemically combined and physically adsorbed water exists in the solid phase or as solid solution, can it be utilized by plants? The answer to this question may be obtained from the following facts: (1) This chemically combined and physically adsorbed water, although probably in the solid phase, is not in an absolutely unchangeable and unutilizable condition; it will evaporate almost like ordinary liquid water; it will become free by certain treatments; and it will be adsorbed by solid substances which exert an attraction for it, etc. (2) It has been found that plants will obtain water from ice (7), which is a solid solution. (3) In conducting soil-moisture studies Alway (1) and other investigators have found that plants under certain conditions can reduce the moisture content in the soil near the hygroscopic moisture content, and consequently below the wilting coefficient.

From these considerations, therefore, it appears that plants can and do utilize this unfree water which is conceived to exist as physically adsorbed and loosely chemically combined, probably in the solid phase.

On the other hand, the water in this condition must be held in the soil with a considerable force and the plants can obtain it only at an

exceedingly slow rate. If the intensity of the atmospheric evaporation is greater than the velocity with which the water is extracted from the soil and absorbed, the plants will wilt. The wilting of plants, therefore, appears to be due mainly to two factors: (1) To the large force with which the unfree water is held in the soil, and (2) to the great osmotic pressure of the soil solution at and below the wilting coefficient. As has been shown in other publications (2), the osmotic pressure of the soil solution of the complex types of soil slightly above the wilting coefficient amounts to 15 atmospheres, and its magnitude increases tremendously with small decreases in the moisture content.

The foregoing views that the soil moisture at the lower magnitudes of water content existing in the solid phase is due to the chemical and physical absorbing forces, and that the wilting of plants is due mainly to the great force with which this water is held in the soil and also to the large osmotic pressure of the soil solution, are contrary to the prevalent views upon the subjects. In the first place, it is quite generally believed that the water in the soil at the lower magnitude of moisture content and even below the hygroscopic coefficient exists as films in the liquid phase. That this is the common view is indicated by the following facts: (1) The movement of moisture is attributed to the curvature of the capillary films. (2) Attempts have been made to measure the thickness of these films around the soil particles or floccules; the crumb structure in the soil is attributed to the force of the water films, etc.

In the second place, the wilting of plants is attributed by some investigators mainly to the slow movement of the moisture in the soil. This view is well expressed by Shull (9) in a recent article. He says (p. 28):

> The view is held, therefore, that the wilting at this critical soil moisture content must be due to the increasing slowness of the water movement from soil particle to soil particle, and from these to the root hairs, the rate of movement falling below that necessary to maintain turgidity of the cells of the aerial parts, even under conditions of low transpiration.

Shull came to this conclusion from a comparison between the force with which the moisture is held in the soil at the wilting coefficient and the osmotic pressure of the sap or root cells. He measured the force with which the soils retain moisture by means of Xanthium seeds. He found that at the wilting coefficient this force amounts to only four atmospheres. The osmotic pressure of the sap of root cells amounts to about seven or eight atmospheres or twice as much as the force of the soil. From these data he logically concluded, therefore, that at the wilting coefficient there is still plenty of moisture and a gradient for the movement of water toward the plant, and yet the plants wilt; this wilting, therefore, is due to the slow movement of the soil moisture.

The magnitude of the force with which the moisture is held in the soil at the wilting coefficient, according to the method of Shull, is not confirmed by the results obtained by the freezing-point method and the

dilatometer. As has already been stated, the osmotic pressure of the soil solution alone is about 15 atmospheres, slightly above the wilting coefficient. Now, if the greatest portion of the moisture at this point exists as physically adsorbed and loosely chemically combined water probably in the solid phase, then this water must be held in the soil with considerable force, and this force must be added to that of the solution alone. The total force with which the moisture is held back in the soil, or the "back pull," according to these estimations, is many times greater than that obtained by Shull.

If, however, the wilting of plants is due to the slow movement of water, then why does not this water freeze if it is in the liquid phase and in an available form, since the rate of movement of water does not affect the freezing as it might the wilting of plants?

VALUE OF THE DILATOMETER METHOD

The functions of the dilatometer method may be summarized as follows: The greatest value of the method appears to be (1) in showing that soils cause water to become unfree or inactive, as indicated by its refusal to freeze; (2) in fixing an approximate estimation of the quantity of water thus becoming unfree; (3) in obtaining, under certain conditions, the wilting coefficient of soils very rapidly; and (4) in classifying, under certain arbitrary conditions, the water in the soil into free, capillary, physically adsorbed, and loosely chemically combined. This method promises to yield future results which will probably revolutionize our present knowledge concerning the moisture in the soil.

In all these functions, therefore, the dilatometer method appears to be of considerable value and importance in yielding knowledge on the fundamental questions regarding the effect of the soil upon the soil moisture and the condition in which the moisture exists in the soil.

SUMMARY

In the present paper there is presented the dilatometer method as a means of showing the amount of water which the soils cause to become unfree or inactive, as indicated by the quantity that fails to freeze.

The principle of the dilatometer method is based upon the fact that water expands upon freezing. If the amount of expansion that a given quantity of water, 1 gm., produces upon freezing is known, the total amount of water that freezes in a soil can be calculated. If also the total water content of the soil is known, the amount of water that does not freeze can be obtained by difference.

The dilatometer (fig. 1) consists of three parts: (1) a bulb, (2) a thermometer, and (3) a measuring stem.

The method of procedure consists of mixing soil and water in certain definite proportions, placing this moist soil in the bulb, and then filling

the latter with ligroin. The bulb with its contents is then placed in a cooling mixture and allowed to supercool. After the desired supercooling is attained the bulb is moved gently in the cooling mixture until solidification commences, which is indicated by the rise of the ligroin in the stem. The bulb is allowed to remain in the ice mixture with frequent movement until equilibrium is reached. The total rise of the ligroin in the stem is taken to represent the total quantity of water that freezes in the soil.

It was found that not all of the water added to soils freezes, some of it fails to freeze, and the quantity that fails to freeze is different in the various classes of soil. Under the empirical conditions of 25 gm. of air-dry soil mixed with 5 c. c. of water, supercooled to 3° C. in a temperature of −4°, the quantity that fails to freeze varies from 2 per cent in quartz sand to 80 per cent in clay, of the 5 c. c. of water added. It increases, therefore, from the simple and noncolloidal types to the complex and colloidal types of soil.

In the case of colloidal soils the amount of water that fails to freeze decreases with the increase in supercooling, but in the case of noncolloidal soils it remains the same.

By increasing the degree of moisture content the amount of water that fails to freeze is decreased in the colloidal soils, but remains practically the same in the noncolloidal soils.

At the low-moisture content successive freezings diminish the quantity of the unfrozen water in the case of the colloidal soils, but not in the noncolloidal soils.

The percentage of water content that fails to freeze in all soils under the empirical conditions of 25 gm. of air-dry soil mixed with 5 c. c. of water, supercooled to 3° in a temperature of −4° corresponds remarkably closely to the moisture content known as the wilting coefficient, to the percentage of moisture at which solidification can not be started, to the thermal critical moisture content, etc.

This water which fails to freeze is designated as unfree or inactive water. Its exact condition in the soil is not definitely known. There are many evidences, however, which indicate that a large portion of it may exist as physically adsorbed and loosely chemically combined, probably in the solid phase, or as solid solution. The quantity of the chemically combined probably exceeds that of the physically adsorbed.

This physically adsorbed and chemically combined water, although probably in the solid phase, is not in an absolute unchangeable condition, but it can be converted into free or available water, by various factors or treatments. Its magnitude, therefore, is not absolutely fixed, but varies with the empirical conditions of the method employed for its determination.

The wilting of plants appears to be due mainly to two causes: First, to the great force with which the moisture is held by the soil, and, second, to the large osmotic pressure of the soil solution at the wilting coefficient.

The dilatometer method appears to be of considerable value in showing (1) that soils cause water to become unfree, as indicated by its refusal to freeze, (2) in measuring quantitatively the amount of water thus becoming unfree, (3) in determining, under certain empirical conditions, the wilting coefficient of soils, and (4) in classifying, under certain empirical conditions, the water in the soil into free, capillary, physically adsorbed, and chemically combined.

The work herein reported should be considered only as preliminary. The investigations upon the subject are being continued.

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LEAFSPOT-ROT OF POND LILIES CAUSED BY HELICOSPORIUM NYMPHAEARUM

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INTRODUCTION

About the middle of May, 1913, the attention of this laboratory was drawn to an irregular spotting and decaying of leaves of pond lilies (*Nymphaea* spp.) in the aquatic gardens at Kenilworth, D. C. The disease was first noticed in the greenhouse propagating tanks, where some of the tender lilies were being started prior to setting in the open ponds. In some cases all the leaves succumbed, so that plants thus affected were too weakened for profitable growth during the following summer. As the season advanced, the disease appeared also in the open ponds, upon both the tender and the hardy varieties of pond lilies, and the inroads upon the leaves were so severe as to demand an attempt at control. From the general appearance of the leaf injury and its rapidity of spread from affected leaves it seemed evident that the disease was of a parasitic nature. On account of the severity of the disease in this particular locality and season, the present study was undertaken primarily to test the efficacy of spraying the floating leaves of a water plant with ordinary fungicides. However, the causal fungus itself proved so interesting that considerable time has also been devoted to a study of its characters and relation to the host. As demonstrated by similar signs on the host, isolation of the same fungus, and its successful inoculation into healthy pond-lily leaves, this disease has also been found by the writer at Arlington, N. J., at the Brooklyn Botanical Gardens, Brooklyn, N. Y., and at the New York Botanical Garden, New York City.[1] An apparently identical leaf disease was likewise seen at Riverton, N. J., though no cultures were attempted from this locality. Hitherto, so far as ascertained, no data upon this disease have been published.

SIGNS OF THE DISEASE

The disease first appears in the form of tiny dark specks on the leaf blade. (Pl. 67, *A*). In those varieties with red pigment in the cells of the lower surface, the initial specks are often reddish or bordered with red, as seen from the upper surfaces of the leaves. At first roundish in outline, the spots become more or less irregular with an increase in size until, either individually or by the coalescence of several initial infection

[1] The fungus from the New York Botanical Garden was isolated by Mrs. Ella M. A. Enlows, of the Bureau of Plant Industry.

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areas, they may, in the thinner leaved species, involve the entire leaf. (Pl. 68, *A*, *B*.) These areas are olivaceous black,[1] often somewhat lighter colored in the center, and they present a water-logged appearance. Although often present at first, the reddish border usually disappears with the rapid inroads upon the leaf made by the later stages. Finally in the thin-leaved species the entire blade may become a dark, olivaceous-black sodden mass of tissue which falls apart at the slightest touch. In the thick-leaved species, and especially in the case of older leaves, the spots, after attaining a diameter of 15 to 20 mm., may show no further increase for a considerable time. Such leaves, however, usually succumb in the end. Under conditions favorable for the parasite, the length of life of the host depends only on its capacity repeatedly to send up new leaves.

ISOLATION OF THE CAUSAL FUNGUS

In order to ascertain the nature of the parasite, several leaves of the pond lily (*Nymphaea odorata*) with initial stages of infection were collected from the open ponds at Kenilworth, D. C., in May, 1913. A few of these leaves were washed for three minutes in a 1 to1,000 solution of mercuric chlorid to kill surface organisms, so far as possible. They were then given three washings in sterile distilled water and placed in damp chambers. Another lot of leaves was placed directly in damp chambers without the previous sterilizing and washing. Freehand sections of young leafspots were examined under the microscope and showed sparingly a brownish, septate mycelium in the diseased leaf tissues. The leaves placed directly in damp chambers without washing developed various mold fungi, such as species of Penicillium and Botrytis, which soon covered the surface with a sporulating mass of hyphæ. Although these fungi were evidently saprophytic surface organisms which were outgrowing the real parasite, pure cultures were started for inoculation tests. In the meantime, after four or five days, signs of a fruiting fungus were noted on many of the leaves sterilized before being placed in damp chambers. Small tawny and somewhat powdery areas were seen on the surface of some of the leaf spots, and a microscopical examination showed these small masses to consist of large, many-septate, curved spores borne in clusters. These conidia were larger at the basal end, tapering and more or less helicoid at the apical end. This form alone appeared on the majority of the sporulating spots and gave promise of a causal relation. Corn-meal-agar plate cultures were poured, using these conidia and also using small pieces of leaf tissue from the younger spots. From the conidia single spore cultures were started by locating germinating conidia in the agar plates and transferring these to other plates of solidified sterile corn-meal agar, where the growth could be followed under the microscope. Colonies developing from these single spores were designated as isolation

[1] Ridgway, Robert, Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

205. The same fungus was also obtained in pure culture from pieces of the diseased leaf tissue. Three species of bacteria, one giving yellow and two giving white colonies, a sterile nonseptate fungus, and species of Aspergillus, Penicillium, and Botrytis were also obtained in culture by this method.

July 20–22, 1914.—Leaves of *N. odorata* with early stages of infection were collected in an open pond at the Brooklyn Botanical Gardens, Brooklyn, N. Y.; and two days later similar specimens were collected at Arlington, N. J. These were taken to the laboratory and plate cultures started from bits of diseased leaf tissue after sterilizing and washing the surface of the leaves. In this way a fungus similar to isolation 205 was obtained from each set of material. The fungus obtained from the Arlington material was designated as isolation 217 and that from the Brooklyn material as isolation 220.

August 24, 1914.—Affected leaves of one of the blue lilies were collected from an open pond at Kenilworth, D. C. Isolation by the method described in the preceding paragraph gave a fungus similar to isolation 205, which was designated as isolation 225.

October 30, 1914.—In the same way this fungus was reisolated from affected leaves of *N. odorata* inoculated on October 24, 1914, from a pure culture of isolation 225. This reisolation was designated as isolation 249.

The fungus was isolated in the same way by Mrs. Ella M. A. Enlows from affected leaves of *N. odorata* collected at Kenilworth, D. C. (Isolation En 59, September 6, 1915); and from similar leaves collected at the New York Botanical Garden, New York City (Isolation En 172, July 25, 1916).

The fungus was most readily isolated from young spots from the thicker leaved species of pond lily. In those with thin leaves various accompanying fungi and bacteria were more abundant, due, probably, to the more rapid softening of the tissues and earlier entrance of saprophytes.

INOCULATION EXPERIMENTS

Inoculation tests were made with all these isolations upon healthy leaves of both hardy and tender species of water lily. A part of these tests was made in the laboratory on cut leaves floated in tap water. Tiny spore clusters from corn-meal-agar cultures were scattered about in drops of water on the upper surface of each leaf without abrasion of the epidermis and the glass containers were left covered for a few days to give a moist condition for the germination of the conidia. Under these conditions the leaves are nearly in their normal environment except that the leaf petioles are severed from the parent plant. When not inoculated, these leaves have remained green and turgid for 10 days to 2 weeks or longer.

Unless otherwise stated, leaves for laboratory inoculation were collected from the open ponds at Kenilworth, D. C. Conidia used were from 1- to 2-weeks-old corn-meal-agar cultures, and the 10-inch glass

containers in which the leaves were floated were left covered for three days after inoculation. Control leaves were treated like the inoculated leaves, except that the fungus was not introduced.

Greenhouse inoculations were made upon plants growing in galvanized-iron tubs in one of the department greenhouses. These inoculations were carried out in the same way as in the laboratory. Each leaf was left covered for several days with a shallow bell jar supported on stilts so that the base of the bell jar dipped just below the surface of the water. Details of the inoculation experiments are given below.

LABORATORY, JUNE 14, 1913. ISOLATION 205.—Twelve leaves of *N. odorata* were inoculated on the upper surface, and four leaves, floated upside down, were inoculated on the morphologically lower surface. Four control leaves were not inoculated. At the same time two leaves each were inoculated with the following organisms isolated from diseased leaves: *Aspergillus* sp., *Penicillium* sp., *Botrytis* sp., a sterile nonseptate fungus, and the three types of bacteria. Two days later tiny dark-reddish specks were observed on the leaves in every case where the upper surface had been inoculated with isolation 205, except where parts of the surface of the leaves were several millimeters below the surface of the water. In the latter cases no signs of infection were evident. Leaves inoculated on the lower surfaces, those inoculated with other fungi and bacteria, and the control leaves showed no signs of infection throughout the experiment.

After four days the infected areas were 3 to 4 mm. in diameter, the centers brownish, and the outer parts dark olivaceous black. Deeply submerged parts of leaves were still unharmed by the presence of the fungus. The leaves inoculated on the lower surface were mostly submerged, and no visible infection had occurred, except at a point in one leaf near the surface of the water where a break had occurred in the epidermis. The experiment was run for 10 days with no further change except an increase in size of infection areas until at the close, when the infected leaves were for the most part a mass of decaying tissue. The tests with the other forms of fungi and bacteria were repeated twice and in every case gave a negative result, so that these organisms were discarded and attention devoted henceforth to the form represented by isolation 205.

LABORATORY, JUNE 19, 1914. ISOLATION 205.—Eight leaves of *N. odorata* were inoculated with this isolation. After two days infection had started at the inoculation points. After five days the spots were 3 to 35 mm. in diameter. Each inoculation had taken, and the three controls were perfectly sound.

LABORATORY, JULY 7, 1914. ISOLATION 205.—Inoculations were made on 12 leaves of *N. odorata*, 6 leaves of *N. caerulea* (Pl. 68, *A*, *B*), 6 leaves of *N. tuberosa* (Pl. 67, *A*), and 3 leaves of one of the tender day-blooming blue lilies. After three days tiny infection specks were observed at nearly every spot inoculated. After four days infection had started at all inoculation points. In most cases there were small reddish spots up to the size of a pin head. After a week single spots had increased in area up to 30 and 35 mm. in diameter. The main part of the diseased spots was water-soaked and olivaceous black in color, and in many cases the margins were reddish. No controls were run with this test.

LABORATORY, AUGUST 8, 1914. ISOLATIONS 205 AND 227.—One leaf each of *N. odorata* and *N. daubeniana* was inoculated with isolation 205, and one leaf of each species was held for a control. After four days small reddish spots were visible at the inoculation points on both leaves. After six days the spots had increased considerably in size, and were olivaceous black and water-logged. The controls were sound.

By the same method, inoculations with isolation 205 were also made on four healthy leaves of Egyptian lotus (*Nelumbium speciosum*). In this case the glass con-

tainers were left covered for five days, but after eight days no signs of infection had appeared.

A species of Alternaria obtained from a leafspot of lotus was tested upon four leaves of *N. odorata*. No infection had resulted after eight days.

LABORATORY, AUGUST 8, 1914.—Isolations 217 and 220 were inoculated on four leaves each of *N. odorata*. After four days small infection spots had started on all the inoculated leaves and at the majority of points of inoculation. After eight days the infections had developed into the typical irregular spots. The four control leaves were sound.

LABORATORY, OCTOBER 16, 1914. ISOLATIONS 205 AND 225.—Inoculations were made with isolation 205 on four leaves of *N. odorata*, and one of *N. daubeniana*, and with isolation 225 on three leaves of *N. odorata* and one of *N. zanzibariensis*. These inoculations were from 45-day-old corn-meal-agar cultures of each isolation. In 5 to 6 days all inoculations with 205 had taken. Two out of three leaves inoculated with 225 showed infection but the third, together with the leaf of *N. zanzibariensis*, remained healthy. Five leaves of *N. odorata* and one each of *N. daubeniana* and *N. zanzibariensis* held through the test as controls remained sound. In this case the fungus retained its infective power in culture after being held 45 days at laboratory temperature (20°–25° C.).

LABORATORY, OCTOBER 24, 1914. ISOLATIONS 205 AND 225.—Six leaves of *N. odorata* and three leaves of one of the night-blooming species of *Nymphaea* were inoculated on the upper surface with isolation 205, and two leaves of *N. odorata* with isolation 225. Four leaves of each species were floated upside down in the glass containers and inoculated with isolation 225.

After three days small reddish infection spots were starting at most of the inoculation points in all the leaves inoculated on the upper surface. After six days all these inoculations had taken, giving the typical spots, which were now up to 5 or 6 mm. in diameter. Most of the spots were surrounded by a halo of pale yellowish green tissue grading off to the normal green of the leaf. No fungus mycelium could be found in this outer halo. It seems thus evident that a substance or substances produced by the fungus may diffuse into the living leaf tissues and cause the death of the cells ahead of the actual fungus invasion.

After three days no infections were apparent on the inverted leaves. After five days infection areas were starting at inoculation points at the water surface or where the leaf epidermis was covered only by a very thin film of water. Inoculated portions covered by an appreciable depth of water (for example, several millimeters) still showed no signs of infection although in many cases the spore masses were still resting directly on the leaf epidermis. Infections thus took place where the lower epidermis was not submerged, but much more slowly than where inoculations were made on the upper epidermis.

The four control leaves run with these two sets of inoculations were and remained sound throughout.

On October 30, reisolation of the fungus was made from leaves inoculated October 24 with isolation 225. This reisolation was designated as isolation 249. Subsequent inoculations with this isolation gave typical infections.

GREENHOUSE, NOVEMBER 9, 1914. ISOLATIONS 205, 225, AND 249, USING 2- TO 4-WEEKS-OLD CULTURES.—Inoculations were made with isolation 205 on two upper leaf surfaces each of *N. omarana*, *N. rubra*, *N. dentata*, *N. odorata;* with isolation 225 on *N. rubra*, *N. zanzibariensis*, *N. omarana*, and *N. odorata;* and with isolation 249 on *N. daubeniana* and *N. capensis*. Two tubs containing *N. caerulea* and two containing *N. tuberosa* were retained as controls. All inoculated and control leaves were covered by shallow bell jars for four days after inoculation and in each species two to four leaves were inoculated. After three days evidences of infection were observed on many of the inoculated leaves. After five days every inoculated leaf showed the typical spots,

and, so far as could be seen, infection had occurred at every inoculation point. In the case of leaves with red pigment on the lower surface there was a tendency toward a slight reddish border around the spots as seen from the upper surface.

LABORATORY, NOVEMBER 10, 1914. ISOLATIONS 205, 225, AND 249.—Inoculations were made with isolation 205 on five leaves and with isolations 225 and 249 on two leaves each of *N. odorata*, collected from plants growing in one of the department greenhouses. Typical infection spots were visible in three days on all inoculated leaves. After four days infection areas were evident so far as could be determined at every inoculation point, and these varied in diameter from the size of a pin head up to 20 mm. in diameter. The five control leaves of the same species remained sound.

LABORATORY, DECEMBER 31, 1914. ISOLATIONS 205 AND 225.—Inoculations were made with each isolation on five leaves of *N. capensis* (Pl. 68, *C*) and five leaves were held as controls. These leaves were collected from plants growing in one of the department greenhouses. After four days infections were starting at every inoculation point and after one week the olivaceous-black spots varied from mere specks to spots 20 mm. in diameter. The lighter green halo surrounding the dead area, as noted under a previous test, was much in evidence here. This halo was most prominent in the thinner leaved species of pond lily. The five control leaves remained sound.

LABORATORY, APRIL 16, 1915. ISOLATION 205.—Four leaves of *N. capensis*, one of *N. zanzibariensis*, and two of *N. caerulea* were inoculated from a single 2-months-old corn-meal-agar culture. After three days very numerous pinhead spots were observed on all three species of lily, and several olivaceous-black spots up to 5 mm. in diameter on *N. capensis*. After 5 days some of the leaves of *N. capensis* were a mass of water-soaked decaying tissue. Large water-soaked areas and numerous small spots occurred on leaves of the other two species. The light-green halo was noted around the decayed spots on all three species. The four control leaves showed no signs of infection except that one of them had a small spot at the beginning of the experiment and this continued to develop. Apparently it was a natural infection with the same fungus.

LABORATORY, JUNE 25, 1915. ISOLATION 225. INOCULATED BY MRS. ELLA M. ENLOWS.—One leaf each of *N. odorata*, and *N. zanzibariensis* was inoculated with this isolation. After three days small infection areas from mere specks up to 3 mm. in diameter were observed. The decay progressed rapidly so that after one week the two leaves were a mass of soft-rotted tissue. The three control leaves remained sound.

LABORATORY, NOVEMBER 17, 1915. ISOLATIONS 205, AND EN 59, USING 4-DAY-OLD CORN-MEAL-AGAR CULTURES.—Inoculations were made with 205 on one leaf each of *N. capensis* and *N. omarana;* and with En 59 on one leaf of *N. capensis* and two leaves of *N. omarana*. After two days tiny infection specks were noted at the points inoculated with En 59 on leaves of *N. omarana*. After four days infections had started on all leaves inoculated with each isolation, and after six days the olivaceous-black infection areas from En 59 had in many cases a diameter of 12 to 15 mm., and those from 205 in no case were more than 5 mm. in diameter. A larger number of infection areas had resulted and increase in size of spots was more rapid with isolation En 59 than with 205. Isolation 205 had been carried in culture for over two years, while En 59 was a fresh isolation. Possibly this may account for the difference in virulence.

LABORATORY, DECEMBER 13, 1915. ISOLATION EN 59.—Sclerotia from a 2-weeks-old corn-meal-agar slant were used for inoculating three leaves of *N. omarana* and conidia from the same lot of cultures for inoculating two leaves each of *N. zanzibariensis* and *N. omarana* and three leaves of *N. capensis*. After two days tiny infection spots were noted on the leaves inoculated with conidia, but none on those inoculated with sclerotia. After three days small infection spots were just visible on the three leaves inoculated with sclerotia, while they were as large as 2 mm. in diameter on the

conidial inoculations. Observation after nine days showed typical spots, some of which were 40 mm. in diameter, and so far as could be determined infection had occurred at all points of inoculation. The yellowish halo around the infection areas was prominent in each case.

PARASITISM OF THE FUNGUS

Through the inoculation tests above outlined the parasitism of this fungus to leaves of pond lily (*Nymphaea* spp.) is clearly established. Inoculations were made during three years with six different isolations obtained from four localities on leaves of nine species of pond lily. In one experiment where cultures 45 days old were used, seven only out of nine inoculated leaves developed the disease. However, all other inoculations, including several leaves inoculated from cultures 2 months old, were successful. Thus, out of a total of 135 leaves inoculated on the upper surface during three seasons, 133 leaves developed the disease in many places. In fact, in nearly all cases, so far as could be determined, the diseased spots appeared wherever the conidia were placed.

Inoculations on the lower epidermis of pond-lily leaves were partially successful. These infections when they occurred were slower in starting, but once started the progress of the disease was apparently as rapid as with inoculations from the upper surface.

In the one inoculation test made with this fungus on leaves of Egyptian lotus (*Nelumbium speciosum*) no infection resulted.

CULTURAL CHARACTERS OF THE FUNGUS

The gross characters of the fungus as grown upon several of the common culture media are as follows: Color descriptions are according to Ridgway.[1]

On beef-agar slants a smooth, pale, vinaceous fawn to vinaceous buff, feltlike mat of mycelium soon develops on the surface of the medium. After two to three weeks no further change takes place except a gradual drying of the agar. No sclerotia have been observed, but branching hyphæ with swollen cells are frequent, and somewhat atypical conidia are sparingly developed.

In beef bouillon in test tubes a water-tight felt is formed at the surface of the liquid, the latter remaining clear after several weeks. This mycelial mat is at first whitish but later approaches a pale vinaceous fawn to vinaceous buff color.

On corn-meal-agar slants the growth is at first whitish with scant aerial mycelium. In four to five days conidia begin to develop in the central portion of the colonies, giving a velvety appearance to the surface. In color the sporulating surface is Dresden brown to mummy-brown. After two to three weeks the dark, velvety sporulating portion nearly to quite covers the surface of the slant and the medium to the depth of several millimeters gradually assumes a light purplish-vinaceous to purplish-vinaceous cast which grades off into the pearl-gray of the agar. From this diffusion of the pigment into the medium it is clearly shown to be a water-soluble substance. Black, roundish sclerotia consisting of a solid mass of fungus cells often develop in old corn-meal-agar cultures (Pl. 67, *C–E*).

[1] Ridgway, Robert. Op. cit.

On cooked white corn meal in flasks the mycelial growth is feltlike with somewhat cottony, pale purplish vinaceous to light pinkish cinnamon aerial mycelium at the surface of the medium. The sporulating parts are a Dresden brown to mummy-brown, while the sclerotial masses in old cultures approach a deep neutral-gray. Conidia are produced rather sparingly, but rounded to irregular black carbonaceous sclerotia are produced abundantly after two to three weeks' growth.

In lavender-blue litmus milk in test tubes a surface felt is formed with no growth in the lower part of the tube. Around the edge of the tubes the felt is slightly paler than pale purplish vinaceous with cinnamon to orange-cinnamon rings; while over the upper surface it approaches Dresden brown to mummy-brown. The casein is gradually precipitated, leaving a dark purplish supernatant fluid. The part above this shows no change in color when compared with check tubes, but bluish rings finally develop around the margin of the surface growth.

On oxalic-acid-agar slants the surface mycelium at first approaches light purplish vinaceous, later becoming fawn color. The sporulating surface is nearly avellaneous. Conidia are sparingly developed, but are not as uniform in size or shape as on corn-meal agar. The red color of the medium (due to addition of neutral-red) becomes gradually bleached out, until in three to four weeks it assumes the color of plain beef agar.

On potato-agar slants a Tilleul-buff cottony mycelium develops within a few days over the surface of the agar. A moderate number of typical conidia are borne on the finally somewhat feltlike mycelium. Even after two or three weeks there is no further increase in the number of spores borne. No sclerotia have been noted.

On cooked potato cylinders the appearance is somewhat similar to that on potato agar. The loose feltlike growth is Tilleul buff to vinaceous-buff in color. No conidia or sclerotia have been observed even after three to four weeks.

On cooked rice in test tubes a feltlike growth of shell-pink to buff-pink mycelium is formed. In four to seven days spores begin to develop, giving the surface a shade closely approaching Saccardo's umber. Conidia and sclerotia are fairly abundant, but the latter are not so regular in shape as on cooked string beans.

On cooked string beans the cottony mycelium covering the surface at first is a Tilleul buff varying slightly in shade on both sides of this color. The sporulating surface developed after four to ten days approximates a dark mouse-gray. Typical conidia are abundantly developed, and after one to three weeks there appear considerable numbers of roundish sclerotia. These have all the external appearances of pycnidia or perithecia, but on breaking upon a slide, or sectioning, these were invariably found to consist of a solid mass of fungous cells, and no spores of any kind were to be found.

On synthetic agar [1] slants a thin feltlike mycelial weft covers the surface in a few days. This weft corresponds almost exactly to the shell pink of Ridgway's chart.[2] After about three weeks' growth the agar, which is a very dark brown at first, becomes bleached to about the color of plain beef agar. No spores, sclerotia, or swollen cells are developed on this medium; but numerous round, highly refractive bodies occur in the hyphæ.

[1] (1) 1,500 c. c. of distilled water and 36 gm. of agar. Cook in double boiler for one hour at 15 pounds' pressure.

(2) 500 c. c. of distilled water, 200 gm. of dextrose. 40 gm. of peptone, 20 gm. of ammonium nitrate, 5 gm. of magnesium sulphate (crystals), 10 gm. of potassium nitrate, 5 gm. of potassium acid phosphate (K_2HPO_4) and 0.2 gm. of sodium chlorid.

Boil in double boiler for 30 minutes, add agar, and cook for five minutes. Restore to volume, titrate, cool to 60° C., and add whites of two eggs. Cook to coagulate eggs; filter, tube, and sterilize.

This formula is modified from that given by Darwin and Acton. (Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. ed. 3, p. 68. Cambridge, [Eng.], 1901.)

[2] Ridgway, Robert. Op. cit.

VIABILITY OF THE FUNGUS IN CULTURE

In order to ascertain the viability of the fungus in culture under ordinary laboratory conditions transfers to corn-meal-agar slants were made from time to time during two years, and plates poured from old corn-meal-agar cultures. Tests were made of cultures 11 months old. None of this age were viable. With strains 205 and 225 three tests each were made at different times with cultures 3½, 4, 5, and 7 months old, respectively; and with 249, three tests with cultures 3½ months old, and one test each with cultures 5 and 7 months old. At the same time that transfers were made to corn-meal-agar slants, corn-meal-agar plates were poured from each of these old cultures so that germination could be followed under the microscope (Pl. 69, *A*). Up to seven months every culture tested gave growth readily on corn-meal-agar slants and in petri dishes, and the colonies developed the typical conidia in abundance. Examination of the petri-dish cultures showed that germination occurred from both conidia and sclerotia. Two successful sets of inoculations were made on pond-lily leaves directly from cultures 45 days and 60 days old, respectively. In addition, transfers from a few of the 3½, 4, 5, and 7 months old cultures were tested by inoculation on leaves of pond lily; and in each case typical infection resulted. This, together with microscopical examination, showed the colonies to be from the original fungus and not from an intruder. This would tend to show that the conidia and sclerotia are both capable of carrying the fungus over for a considerable period of time under certain adverse conditions, such as drying and increased acidity of the medium, at temperatures ranging from 20° to 30° C.

SCLEROTIAL GERMINATIONS

Sclerotia from 1-, 3-, 6-, 8-, 9-, and 11-months-old corn-meal-agar cultures were sown on sterile moistened sand and sterile moistened garden soil and held at laboratory temperature (about 20° C.). Germination took place from all the sclerotia, except in the case of those from the 11-months-old cultures, but microscopical examination at intervals during three weeks failed to show any sporulating form in connection with them. Sections after two and three weeks showed the sclerotia still made up of a solid mass of fungus cells, hyalin in the interior and brownish and thick-walled on the exterior. This is the structure shown by both young and old sclerotia in culture (Pl. 67, *C*, *E*), and in no case have evidences of a pycnidial or perithecial stage been observed.

Single sclerotia separated from mycelium and conidia and sown in fresh culture media readily send out germ tubes from the superficial cells (Pl. 67, *D*), and successful inoculations to leaves have been made, using such isolated sclerotia.

TEMPERATURE TESTS

Of 80 transfers of two isolations of the fungus to corn-meal-agar slants 40 were placed at once in a 10-compartment refrigerator with temperatures ranging from 2° to 19° C. The other half were placed in the compartments the following day, after the conidia had germinated. Where germination had occurred before placing cultures in the refrigerator, a very slight growth took place at 2°, but no perceptible growth occurred where transfers were held from the start at this temperature. Slight mycelial growth took place at 4° to 5° in all tubes with increase in rapidity following a rise in temperature. Development of conidia began at 8° to 9° and increased in rapidity up to the highest temperature tested. Sclerotia began to develop at 8° to 9°, increased in numbers up to 14° to 15°, and declined in numbers up to 19° to 20°, where but few were formed. All cultures held at the lower temperatures developed rapidly when later placed at 18° to 20°. These observations covered a period of five weeks.

HOST-PARASITE RELATION

Numerous microscope sections have been made of naturally infected leaves and also of artificially infected leaves at periods of time varying from a few hours to several days after inoculation. Study has also been made of portions of leaves killed, bleached, and stained without sectioning at 18, 24, 48, and 72 hours after sowing the conidia on the upper surface, as in the inoculation experiments previously detailed. In the older spots the tissues are seen to have more or less completely collapsed and fungus mycelia are found ramifying both between and within the decaying cells, together with coccoid and rod-shaped bacteria and various protozoa, a condition to be expected in a decaying mass of tissue floating on a watery medium. In the case of leaves studied without sectioning, germination of the conidia has been noted on the leaf surfaces at 18 and 24 hours after inoculation, and in several cases at 24 to 48 hours the germ tubes were seen entering a stomatal opening. By focusing down the continuation of the hyphæ was also seen below the guard cells and epidermal cells in the substomatal cavity.

Furthermore sections of leaf spots at three to four days after inoculation have shown in numerous instances the hyphæ entering the stomatal opening and branching out in the chamber below (Pl. 70, *B*, *C*). In slightly older spots the pale brownish hyphæ have repeatedly been seen ramifying between the cells of the spongy parenchyma and through the large air chambers, as also between the cells of the palisade tissue. But, except in old decayed and water-logged spots, hyphæ have not been seen within the cells.

It is clear that infection may and does occur through the stomata, but this is very evidently not the only method. Infection occurs,

although somewhat more slowly, when inverted leaves are inoculated on the morphologically lower surface, although stomata are present only on the upper surface.

The usual occurrence around the developing spot of a pale yellowish green halo grading off to the normal green of the leaf has been noted previously (Pl. 67, *A*; 68, *C*). No mycelium has been found in this bordering tissue; and even if sometimes present in the intercellular spaces, it is almost certainly not intracellular. From these facts it is evident that some product or products of the fungus metabolism are capable of diffusing into, injuring, and finally killing the host cells.

The epidermal cells in sections of healthy leaves stained with Flemming's triple stain (Pl. 70, *A*) are seen to be free from coagulation products, the nuclei are clearly differentiated, and the numerous chlorophyll bodies are well rounded out and definite in outline. In the infected areas even at so short a time as three to four days after inoculation the epidermal cells are frequently filled more or less completely with coagulation products, the nuclei are disintegrating, and the chlorophyll bodies are fewer in number, and those left are seen to be disintegrating (Pl. 70, *B*, *C*). The diseased parts of the leaf take the orange stain more readily, while the healthy parts show a decided affinity for the gentian violet. These differences can not be due to variations in individual leaves nor to differences in microtechnic, for where sections have been made through the edge of a young spot into normal tissues beyond it, all these differences have been seen in a single section.

Conidia have been found in one or two cases on freshly collected material and have developed twice on the upper surface of leaf spots in a damp chamber. Usually, however, after the removal of diseased leaves from the water and after they had been placed in a damp chamber, a fine whitish growth of more or less erect hyphæ develops over the infected areas without the formation of conidia. In several instances sclerotia have developed on the leaf spots after the leaves have been placed in a damp chamber.

The fact that infections have not been found to occur regularly on submerged leaves is probably due in part to a lack of sufficient oxygen, since in liquid and solid media growth of the fungus is almost entirely limited to the surface.

TAXONOMY AND DESCRIPTION OF FUNGUS

This pond-lily parasite belongs to the hyphomycetous group of the Fungi Imperfecti. It is to be included in the family Dematiaceae among those forms with dark, loose hyphæ and conidia, but without definite stromata or fruiting bodies. The elongated, transversely septate, more or less helicoid conidia place it in the genus Helicosporium belonging to the small group Helicosporae. Of the two other genera in this group, Helicopsis is without definite mycelium and Helicoma bears muriform conidia.

The following is a brief description of the fungus under discussion:

Helicosporium nymphaearum, n. sp.

Upon leaves of pond lilies (*Nymphaea* spp.), causing initial brown to reddish flecks, developing into irregular olivaceous-black water-soaked spots, which individually or by coalescence may in some cases involve the greater part of the leaf blade.

Mycelium light brown, intercellular, often hyalin in culture, septate, branched, growing readily on ordinary culture media.

Sporophores long and slender and bearing several conidia near the summit (Pl. 69, *D*). Conidia brown, many septate, constricted at the septa, usually somewhat larger toward the basal end, slightly tapering toward the curved or usually helicoid apical end (Pl. 69, *C*, *E*); outer walls thick and with echinulate sculpturings (Pl. 69, *D*, *F*, *G*); cross walls with roundish thin spot or perforation in the center (Pl. 69, *B*); highly variable in size (60 to 190 by 5 to 18μ); not readily produced on diseased leaves in damp chamber but develop in large numbers on several of the ordinary culture media. Sclerotia roundish, subcarbonaceous, develop sparingly on leaves and in culture, 150 to 900μ diameter.

Habitat: Leaves of *Nymphaea* spp. Type specimens from aquatic gardens at Kenilworth, D. C. Specimens also collected from open ponds at Riverton and Arlington, N. J., at the Brooklyn Botanical Gardens, Brooklyn, N. Y., and at the New York Botanical Garden, New York City.

Helicosporium nymphaearum, sp. n.—Maculis in foliis primo minutis fuscis vel subrufis, deinde subnigris, aquosis et irregularibus, interdum conjunctis postremo; mycelio albido-fusco, intercellulari, septato, ramoso; saepe in culturis hyalino, sporophoris longis, tenuibus, multas conidias prope apicem habientibus; conidiis fuscis, multiseptatis, in septis constrictis, in basi et apice plerumque attenuatis; apicibus conidiarum curvatis vel plerumque helicoideis; muris conidiarum densis, echinatis; conidiis 60–190μ × 5–18μ; Sclerotiis subsphaericis, subcarbonaceis 150–900μ.

Hab. in foliis vivis Nymphaeae speciorum, Washington, D. C., Arlington et Riverton, N. J., New York, N. Y. America Borealis.

CONTROL OF THE DISEASE

The control of diseases of aquatic plants has been little studied. In fact, the only account of actual experiments along this line which has come to the attention of the writer consists in a brief account of tests for the control of a leafspot of pond lilies [1] apparently due to a species of Cercospora. No cultural or inoculation experiments with the parasite are mentioned, but a description is given of a single series of experiments with Bordeaux mixture, oxid of copper mixed with sulphur, sulphur alone, and ammoniacal copper carbonate applied by adding to the water in tubs in which the plants were growing. The Bordeaux mixture is described as giving satisfactory results but as being objectionable on account of its appearance on the foliage. Ammoniacal copper carbonate was preferred to the Bordeaux mixture, but it had to be used in weak solution to prevent injury. The sulphur treatments were unsatisfactory. A secondary advantage from use of the fungicides consisted in the destruction of the green algæ. Spraying with Bordeaux mixture is also recommended by Bisset [2] as an efficient control against the leafspot of pond lilies caused by *Cercospora* sp., but no direct experiment is mentioned.

[1] Halsted, B. D. Experiments with water-lily blight. *In* N. J. Agr. Exp. Sta., 17th Ann. Rpt. [1895]/1896, p. 405–407, fig. 52. 1897.

[2] Bisset, Peter. The Book of Water Gardening . . . p. 188–189. New York, 1907.

In the writer's experiments, carried out in the spring and summer of 1913 at Kenilworth, D. C., Bordeaux mixture (3–3–50 formula) and soda-Bordeaux mixture (2–3–100 formula) were used. In both cases 2 pounds of lead arsenate to 50 gallons of spray mixture were used to combat injurious insects, such as leaf channelers.

The first treatment (May 29, 1913) was applied by spraying the respective mixtures upon the leaves with a hand-pump outfit carried in a boat. Two varieties of *N. odorata* and several varieties of the tender day-blooming blue lilies were treated in this way, with an equal area of unsprayed plants of each variety left as control. Five other similar treatments were given on June 3, 10, 14, 19, and 25. The disease had gained a considerable start before the work was undertaken; hence, the control of the disease was not as complete as it might otherwise have been. However, as shown by careful comparisons of sprayed and unsprayed plots (June 25 to 30), together with leaf counts with respect to injury, the plots sprayed with Bordeaux mixture were clearly 50 per cent less injured by the disease than the control plots. In the plots treated with soda-Bordeaux mixture the control of the disease was almost as high, but in this case a slight spray injury of the leaves was observed.

During the following two seasons this treatment was continued by the owner on a commercial scale with satisfactory results. It might be added that the lead-arsenate treatment was found to be highly efficacious in reducing the injury by leaf channelers.

The ordinary Bordeaux mixture was slightly the better of the two mixtures in controlling the disease, and no spray injury resulted from its use. The soda-Bordeaux mixture, though almost as efficient in its control, and possessed of the advantage of not staining the leaves, caused some spray injury at the strength (2–3–100 formula) and frequency used. Obviously, with frequently submerged foliage, treatments must be made at rather close intervals in order to keep the upper surface of the leaves covered with a film of the fungicide. Apparently little or no infection occurred from the lower surface of the leaves.

SUMMARY

About the middle of May, 1913, the attention of the Laboratory of Plant Pathology was drawn to an irregular spotting and decaying of leaves of pond lilies (*Nymphaea* spp.) in the aquatic gardens at Kenilworth, D. C. On account of the severity of the disease in this particular locality and season the present study was undertaken primarily to test the efficacy of spraying the floating leaves of a water plant with ordinary fungicides. However, the causal fungus itself proved so interesting that considerable time has also been devoted to a study of its characters and relation to the host. From the olivaceous-black water-soaked spots

a fungus belonging to the genus Helicosporium has been isolated from leaves collected at Kenilworth, D. C., Arlington, N. J., and New York and Brooklyn, N. Y. The parasitism of this fungus has been demonstrated by successful inoculations made during three years with six different isolations of the fungus on nine species of pond lily (*Nymphaea* spp.). Hitherto, so far as ascertained, no data upon this disease have been published, and the causal fungus is described as a new species under the name *Helicosporium nymphaearum*.

The fungus gains entrance to the host most readily through the stomata, which occur only on the upper leaf surface. Sections of infected leaves show the mycelium ramifying through the intercellular spaces and occasionally between cells which have become separated. Many of the cells of the epidermis and parenchyma become filled with discolored coagulation products and the nuclei and chlorophyll bodies disintegrate. In the older spots the tissues are seen to have more or less completely collapsed and fungus hyphæ are found ramifying both between and within the decaying cells together with bacteria and various protozoa, a condition to be expected in a decaying mass of tissue floating on a watery medium. The sclerotia and multiseptate conidia are developed sparingly on the diseased leaves and rather abundantly on many of the common culture media.

Spraying experiments with ordinary Bordeaux mixture and with soda-Bordeaux mixture were carried out during one season. The disease had gained a considerable start before the work was undertaken; hence, the control of the disease was not as complete as it might otherwise have been. However, as shown by careful comparisons of sprayed and unsprayed plots, the leaves sprayed with Bordeaux mixture were clearly 50 per cent less injured by the disease than were the unsprayed leaves. The control by the soda-Bordeaux mixture was almost as high, but in this case a slight spray injury of the leaves was observed. During the following two seasons this treatment was continued by the owner on a commercial scale with satisfactory results.

PLATE 67

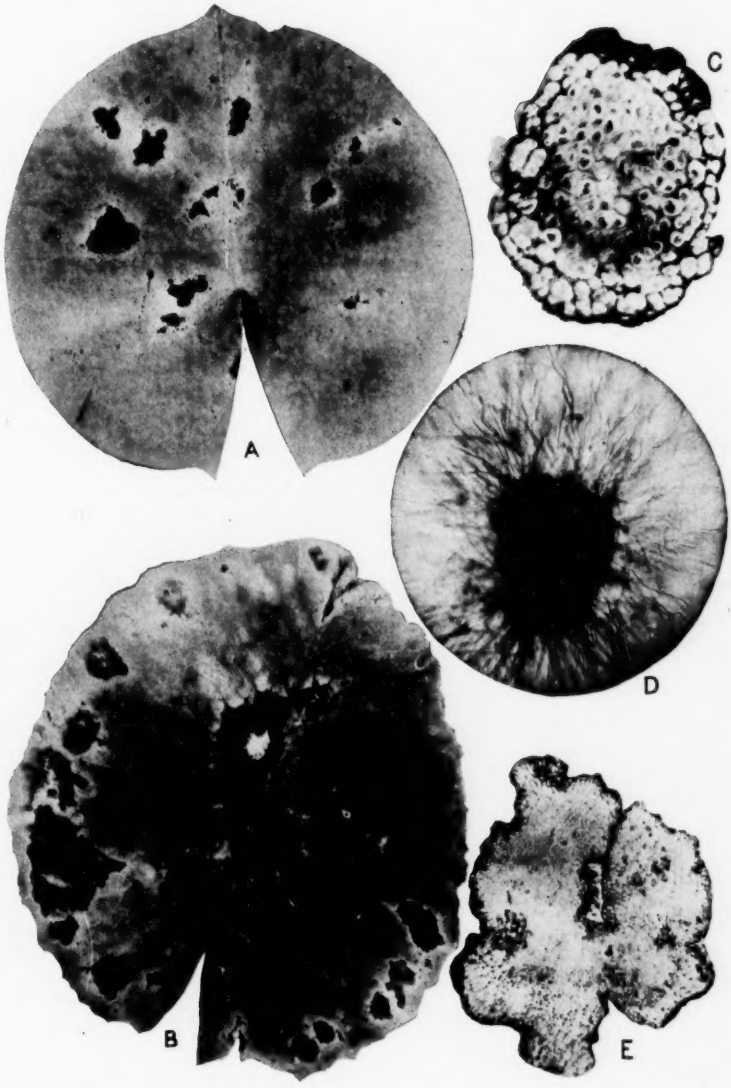
A.—Leaf of *Nymphaea tuberosa* at 1 week after sowing conidia of *Helicosporium nymphaearum* (isolation 205) on upper surface. Note lighter halo around diseased spots. Inoculation of July 7, 1914. ×0.37. Photographed by J. F. Brewer.

B.—Leaf of *Nymphaea* sp. showing spots due to natural infection with *Helicosporium nymphaearum*. ×0.37. Photographed by J. F. Brewer.

C.—Cross section of young sclerotium of *Helicosporium nymphaearum* from a corn-meal-agar culture. ×200. Photomicrographed by J. F. Brewer.

D.—Sclerotium of *H. nymphaearum* from corn-meal-agar culture kept at laboratory temperature for 7 months. Showing germination at 12 hours after sowing in the petri dish of corn-meal agar. ×30. Photomicrographed by J. F. Brewer.

E.—Cross section of old sclerotium developed in a corn-meal-agar slant culture. ×60. Photomicrographed by J. F. Brewer.


A
B
C
D
E

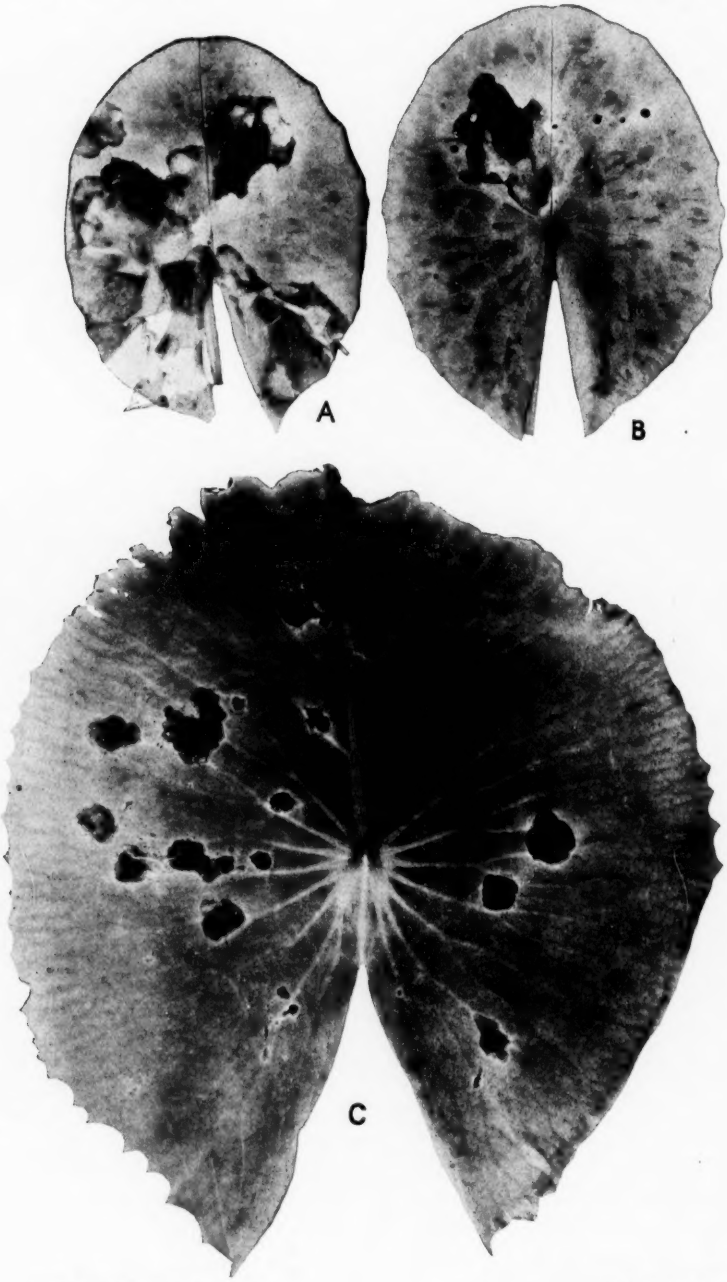

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PLATE 68

A, B.—Leaves of *Nymphaea caerulea* at 1 week after sowing conidia of ***Helicosporium nymphaearum*** (isolation 205) on upper surface. Inoculation of July 7, 1914. × 0.75. Photographed by J. F. Brewer.

C.—Leaf of *N. capensis* at 1 week after sowing conidia of *H. nymphaearum* (isolation 225) on upper surface. Inoculation of December 31, 1914. × 0.77. Photographed by J. F. Brewer.

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PLATE 69

A.—Conidium of *Helicosporium nymphaearum* (isolation 205) from culture 3½ months old showing germination in corn-meal-agar petri dish after 12 hours. ×242. Photomicrographed by J. F. Brewer.

B.—Cross section through conidium of *H. nymphaearum* showing thin spot or perforation in cross wall and surface sculpturings on outer wall. ×900. Photomicrographed by J. F. Brewer.

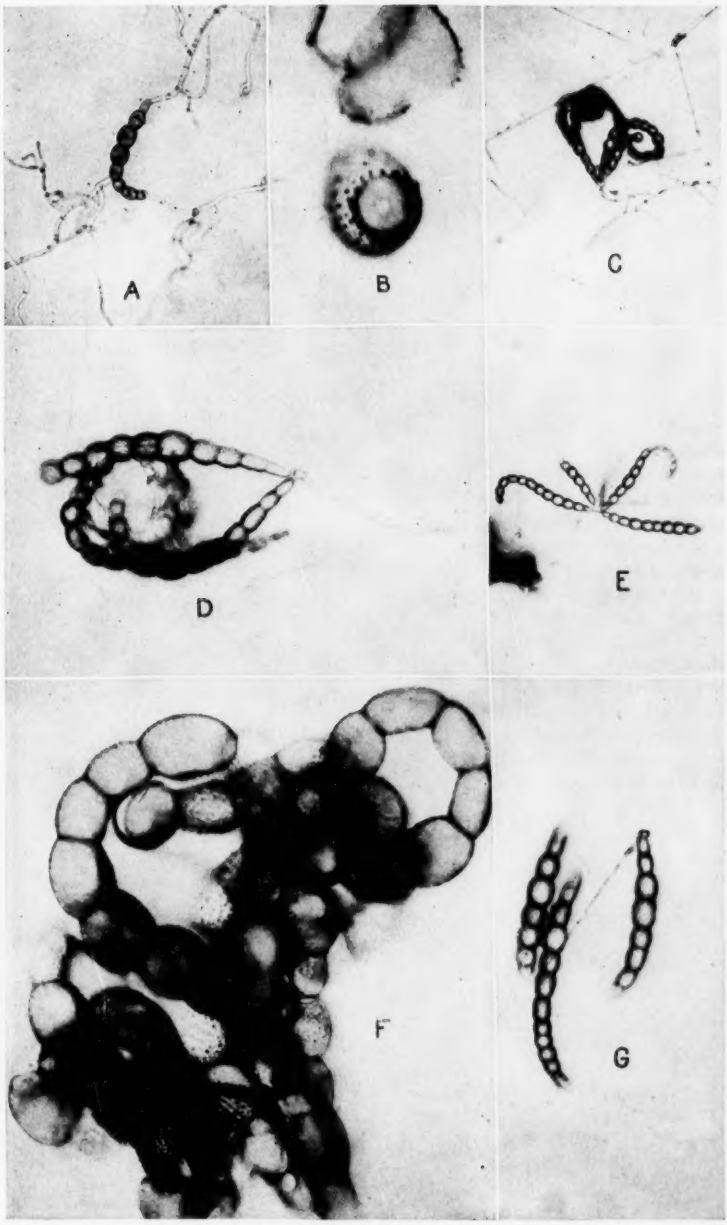
C.—Conidial group (*H. nymphaearum*) *in situ* in petri-dish culture of corn-meal agar at 2 weeks. ×107. Photomicrographed by J. F. Brewer.

D.—Conidia of *H. nymphaearum* showing attachment to sporophore and sculpturings on the outer walls. ×285. Photomicrographed by J. F. Brewer.

E.—Conidial group (*H. nymphaearum*) in corn-meal-agar petri-dish culture at 13 days. ×107. Photomicrographed by J. F. Brewer.

F.—Water mount of conidia of *H. nymphaearum* from a corn-meal-agar petri-dish culture 13 days old, showing echinulate sculpturings on outer spore walls. ×828. Photomicrographed with oil-immersion lens by Dr. Erwin F. Smith.

G.—Conidia of *H. nymphaearum* from corn-meal-agar culture showing sculpturings on outer spore walls. ×285. Photomicrographed by J. F. Brewer.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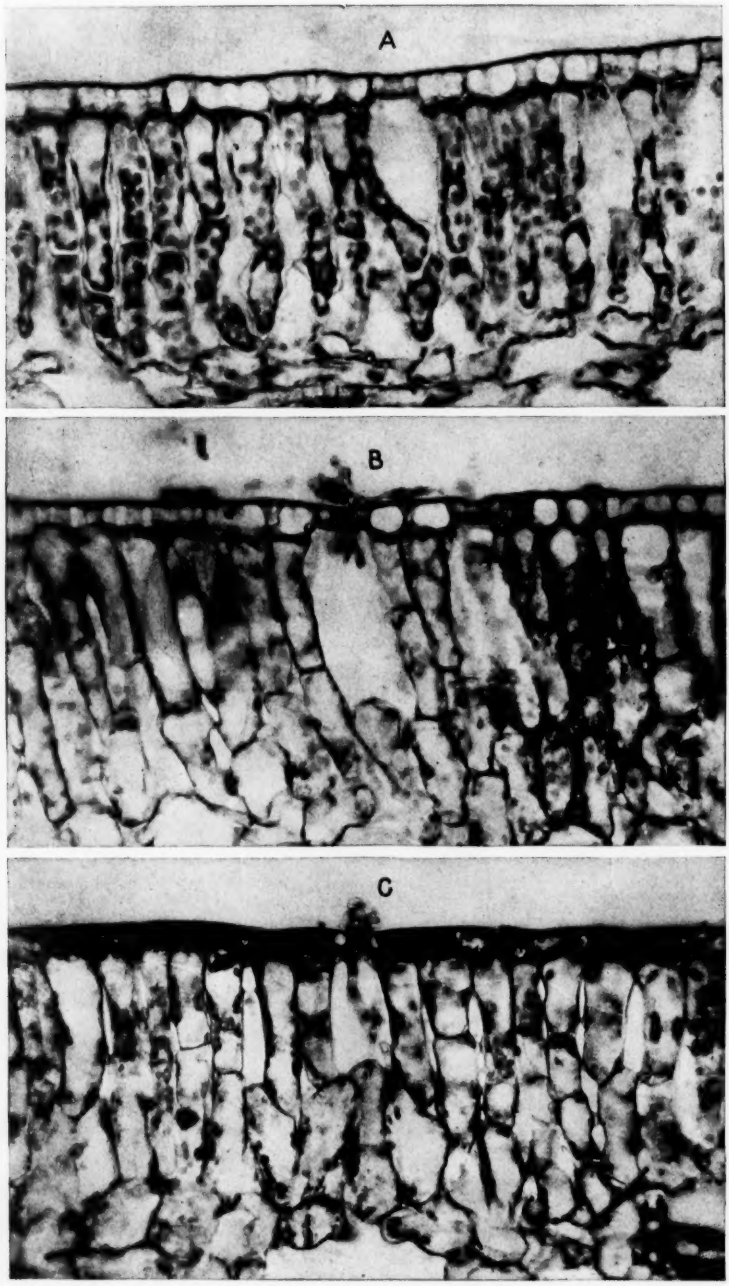
A
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C

PLATE 70

A.—Cross section of normal part of leaf blade of *Nymphaea odorata* the diseased part of which is shown in B. ×430. Photomicrographed by J. F. Brewer.

B.—Cross section of leaf of *N. odorata* at four days after sowing of conidia of *Helicosporium nymphaearum* from pure culture to upper surface of leaf. Note penetration of stomatal opening by germ tube of the fungus. ×430. Photomicrographed by J. F. Brewer.

C.—Cross section of leaf of *N. odorata* at four days after sowing of conidia of *H. nymphaearum* from pure culture to upper surface of leaf. Showing penetration of fungus through stomatal opening and further growth of hyphæ in substomatal chamber. ×430. Photomicrographed by J. F. Brewer.

Compare figures A to C for progressive disintegration of cellular contents of host after entry of the fungus, coagulation of contents of epidermal cells and disintegration of nuclei and chlorophyll bodies.